

**IMPROVED KETOREDUCTASE POLYPEPTIDES FOR THE STEREOSELECTIVE
PRODUCTION OF (R)-3-HYDROXYTHIOLANE**

1. CROSS REFERENCES TO RELATED APPLICATIONS

[0001] This application claims benefit under 35 U.S.C. § 119(e) of application Serial No. 60/957,974, filed August 24, 2007, the contents of which are incorporated herein by reference.

2. TECHNICAL FIELD

[0002] The present disclosure relates to engineered polypeptides and uses of the polypeptides.

3. REFERENCE TO SEQUENCE LISTING, TABLE OR COMPUTER PROGRAM

[0003] The Sequence Listing concurrently submitted herewith under 37 C.F.R. § 1.821 via EFS-Web in a computer readable form (CRF) as file name 376247-016.txt is herein incorporated by reference in its entirety. The electronic copy of the Sequence Listing was created on August 24, 2008, with a file size of 272 Kbytes.

4. BACKGROUND

[0004] Enzymes belonging to the ketoreductase (KRED) or carbonyl reductase class (EC1.1.1.184) are useful for the synthesis of optically active alcohols from the corresponding prostereoisomeric ketone substrate. KREDs typically convert a ketone or aldehyde substrate to the corresponding alcohol product, but may also catalyze the reverse reaction, oxidation of an alcohol substrate to the corresponding ketone/aldehyde product. The reduction of ketones and aldehydes, and the oxidation of alcohols by enzymes such as KRED requires a co-factor, most commonly reduced nicotinamide adenine dinucleotide (NADH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH), and nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP) for the oxidation reaction. NADH and NADPH serve as electron donors, while NAD and NADP serve as electron acceptors. It is frequently observed that ketoreductases and alcohol dehydrogenases accept either the phosphorylated or the non-phosphorylated co-factor (in its oxidized and reduced state).

[0005] KRED enzymes can be found in a wide range of bacteria and yeasts (for reviews: Kraus and Waldman, *Enzyme catalysis in organic synthesis* Vols. 1&2. VCH Weinheim 1995; Faber, K., *Biotransformations in organic chemistry*, 4th Ed. Springer, Berlin Heidelberg New York. 2000; Hummel and Kula, 1989, *Eur. J. Biochem.* 184:1-13). Several KRED genes and enzyme sequences have been reported, e.g., *Candida magnoliae* (Genbank Acc. No. JC7338; GI:11360538) *Candida*

parapsilosis (Genbank Acc. No. BAA24528.1; GI:2815409), *Sporobolomyces salmonicolor* (Genbank Acc. No. AF160799; GI:6539734).

[0006] In order to circumvent many chemical synthetic procedures for the production of key compounds, ketoreductases are being increasingly employed for the enzymatic conversion of different keto substrates to chiral alcohol products. These applications can employ whole cells expressing the ketoreductase for biocatalytic ketone and aldehyde reductions, or purified enzymes in those instances where presence of multiple ketoreductases in whole cells would adversely affect the stereopurity and yield of the desired product. For *in vitro* applications, a co-factor (NADH or NADPH) regenerating enzyme such as glucose dehydrogenase (GDH), formate dehydrogenase etc., is used in conjunction with the ketoreductase. Examples using ketoreductases to generate useful chemical compounds include asymmetric reduction of 4-chloroacetoacetate esters (Zhou, *J. Am. Chem. Soc.*, 1983, 105:5925-5926; Santaniello, *J. Chem. Res. (S)* 1984:132-133; U.S. Patent Nos. 5,559,030; US Patent No. 5,700,670 and US Patent No. 5,891,685), reduction of dioxocarboxylic acids (e.g., US Patent No. 6,399,339), reduction of tert-butyl (S) chloro-5-hydroxy-3-oxohexanoate (e.g., U.S. Patent No. 6,645,746 and WO 01/40450), reduction pyrrolotriazine-based compounds (e.g., US application No. 2006/0286646); reduction of substituted acetophenones (e.g., US Patent No. 6,800,477); and reduction of ketothiolanes (WO 2005/054491).

[0007] It is desirable to identify other ketoreductase enzymes that can be used to carryout conversion of various keto and aldehyde substrates to its corresponding chiral alcohol products.

5. SUMMARY

[0008] The present disclosure provides ketoreductase polypeptides having the ability to reduce 3-ketothiolane (hereafter referred to as "the substrate") to (*R*)-3-hydroxythiolane (hereafter referred to as "the product"), the polynucleotides encoding such polypeptides, and methods for using the polypeptides. Generally, the engineered ketoreductase polypeptides of the disclosure have an improved property as compared to the naturally-occurring wild-type ketoreductase enzymes obtained from *Lactobacillus kefir* ("*L. kefir*"; SEQ ID NO:4), *Lactobacillus brevis* ("*L. brevis*"; SEQ ID NO:2), and *Lactobacillus minor* ("*L. minor*"; SEQ ID NO:142) in converting the defined keto substrate to the corresponding chiral alcohol product. Improvements in enzyme activity can include increases in stereoselectivity, enzymatic activity, thermostability, solvent stability, reduced product inhibition, or combinations thereof.

[0008a] In one aspect, there is provided a ketoreductase polypeptide capable of stereoselectively reducing a 3-ketothiolane to

(R)-3-hydroxythiolane with a percent stereomeric excess of at least 70%, which comprises an amino acid sequence that is at least 85% identical to a reference sequence based on SEQ ID NO:2 or 4 or 142 having at the residue corresponding to X145 a serine, and in which the ketoreductase polypeptide amino acid sequence has at the residue corresponding to X145 a serine.

[0008b] In another aspect, there is provided a polynucleotide encoding a polypeptide as defined above.

[0008c] In another aspect, there is provided an expression vector comprising the polynucleotide as defined above operably linked to at least one control sequence suitable for directing expression in a host cell.

[0008d] In another aspect, there is provided a host cell comprising the expression vector as defined above.

[0008e] In another aspect, there is provided a method for stereoselectively reducing substrate 3-ketothiolane to product (R)-3-hydroxythiolane, which comprises contacting the 3-ketothiolane with the ketoreductase polypeptide as defined above under suitable reactions conditions for reducing or converting the 3-ketothiolase to the (R)-3-hydroxythiolane.

[0008f] In another aspect, there is provided a composition comprising the compound 3-ketothiolane and a ketoreductase as defined above.

[0009] In some embodiments, the ketoreductase polypeptides of the disclosure comprise an amino acid sequence in which the amino acid residue corresponding to position 145 of a reference sequence of SEQ ID NO:2, 4 or 142 is not an acidic residue, *i.e.*, glutamic acid or aspartic acid. As such, the

residue corresponding to position 145 is a non-acidic residue. In some embodiments, the ketoreductase polypeptides of the disclosure have an amino acid sequence in which the residue corresponding to position 145 is a polar residue. In some embodiments, this residue corresponding to X145 is a serine.

[0010] In some embodiments, the ketoreductase polypeptides of the disclosure have an amino acid sequence that is at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to a reference sequence based on SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:142 (or a region or domain thereof, such as residues 90-211) having at the residue corresponding to X145 a serine, with the proviso that the ketoreductase polypeptide amino acid sequence has at the residue corresponding to X145 a polar residue. In some embodiments, the residue corresponding to residue X145 is a serine. In some embodiments, the ketoreductase polypeptide is based on the sequence formulas of SEQ ID NO:143, 144, or 145, or a domain thereof, such as residues 90-211, in which the residue corresponding to X145 is a polar residue, particularly serine. In some embodiments, the ketoreductase polypeptides can additionally have one or more amino acid residue differences in the amino acid sequence, or the defined domain or region, as compared to the reference sequence, such as the reference sequences of SEQ ID NO:4, 2, or 142. In some embodiments, the amino acid sequence differences in the domain can comprise non-conservative, conservative, as well as a combination of non-conservative and conservative amino acid substitutions. Various amino acid residue positions where such changes can be made are described herein.

[0011] In some embodiments, the ketoreductase polypeptides described herein are capable of stereoselectively reducing the substrate to the product with at a higher stereomeric excess than the wildtype enzyme from *L. kefir* (i.e., SEQ ID NO:4). In some embodiments, the ketoreductase polypeptides described herein are capable of stereoselectively reducing the substrate ketothiolane to the product (*R*)-3-hydroxythiolane with at least about 70% stereomeric excess.

[0012] In some embodiments, the disclosure provides highly stereoselective ketoreductase polypeptides that can reduce the substrate to the product in greater than about 90% stereomeric excess (s.e.). Exemplary ketoreductase polypeptides with such high stereoselectivity include, but are not limited to, the polypeptides comprising the amino acid sequences corresponding to SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 86, 88, 90, 92, 94, 96, 100, 102, 104, 106, 108, 110, 112, 126, 128, 130, and 134.

[0013] In some embodiments, the disclosure provides stereoselective ketoreductase polypeptides that can reduce the substrate to the product in greater than about 98% s.e. Exemplary polypeptides with

such stereoselectivity include, but are not limited to, the polypeptides comprising the amino acid sequences corresponding to SEQ ID NO: 6, 8, 10, 18, 20, 22, 24, 26, 28, 30, 34, 36, 38, 40, 42, 50, 52, 54, 58, 62, 66, 70, 72, 76, 78, 80, and 134.

[0014] In some embodiments, the engineered ketoreductase polypeptide can have increased enzymatic activity as compared to the wild-type ketoreductase enzyme in reducing the defined keto substrate to the product. The amount of the improvement can range from 1.5 times the enzymatic activity of the corresponding wild-type ketoreductase enzyme, to as much as 2 times, 5 times, 10 times, 20 times, 25 times, 50 times, 75 times, 100 times, or more enzymatic activity. In specific embodiments, the engineered ketoreductase enzyme exhibits improved enzymatic activity in the range of 1.5 to 50 times, 1.5 to 100 times greater than that of the wild-type ketoreductase enzyme. Exemplary polypeptides that are capable of converting the substrate to the product at a rate that is improved over wild-type enzyme, include but are not limited to, polypeptides comprising the amino acid sequences corresponding to SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 64, 66, 68, 70, 74, 76, 78, 80, 86, 88, 90, 92, 104, 106, 110, 112, 124, 126, 130, and 134.

[0015] In some embodiments, the disclosure provides ketoreductase polypeptides that have improved activity and stability over the wild-type enzyme, and can reduce the substrate to the product in greater than about 95% s.e. Exemplary polypeptides with such capabilities include, but are not limited to, polypeptides comprising the amino acid sequences corresponding to SEQ ID NO: 6, 8, 12, 14, 22, 24, 26, 30, 32, 38, 42, 44, 46, 50, 52, 56, 58, 60, 64, 66, 68, 70, 74, 76, 78, 80, 82, 86, 88, 90, 92, 104, 106, 110, 112, and 134.

[0016] In some embodiments, the ketoreductase polypeptides of the disclosure are improved as compared to wild-type with respect to their thermostability, as determined by an increase in the rate of enzymatic activity as compared to wild-type under high temperatures. Exemplary ketoreductase polypeptides with improved stability, include but are not limited to, polypeptides comprising amino acid sequences corresponding to SEQ ID NO: 6, 8, 12, 14, 22, 24, 26, 32, 34, 36, 38, 42, 44, 46, 50, 52, 56, 58, 60, 64, 66, 68, 70, 74, 76, 78, 80, 82, 86, 88, 90, 92, 104, 106, 110, 112, 124, and 134.

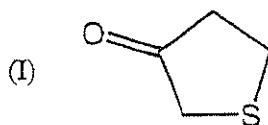
[0017] In another embodiment, the present disclosure provides polynucleotides encoding the engineered ketoreductases described herein or polynucleotides that hybridize to such polynucleotides under highly stringent conditions. The polynucleotide can include promoters and other regulatory elements useful for expression of the encoded engineered ketoreductase, and can utilize codons optimized for specific desired expression systems. Exemplary polynucleotides include, but are not limited to, the nucleotide sequences corresponding to SEQ ID NO: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31,

33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, and 133.

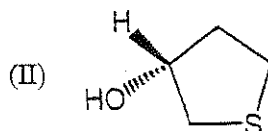
[0018] In another embodiment, the present disclosure provides host cells comprising the polynucleotides and/or expression vectors for purposes of manipulation and expression of the ketoreductase polypeptides. The host cells may be *L. kefir*, *L. brevis*, or *L. minor*, or they may be a different organism. The host cells can be used for the expression and isolation of the engineered ketoreductase enzymes described herein, or, alternatively, they can be used directly for the conversion of the 3-ketothiolane substrate to the chiral (*R*)-3-hydroxythiolane product.

[0019] Whether carrying out the method with whole cells, cell extracts or purified ketoreductase enzymes, a single ketoreductase enzyme may be used or, alternatively, mixtures of two or more ketoreductase enzymes may be used.

[0020] As noted above, the ketoreductase enzymes described herein are capable of catalyzing the reduction reaction of the keto group in the compound of structural formula (I), 3-ketothiolane,



to the corresponding chiral alcohol product of structural formula (II), (*R*)-3-hydroxythiolane,



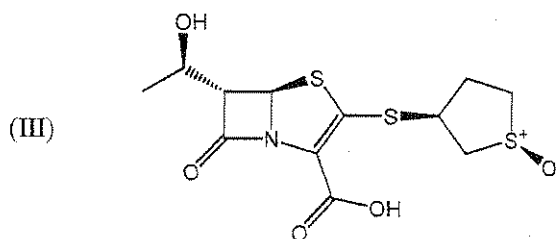
[0021] As such, in some embodiments, the present disclosure provides a method for reducing 3-ketothiolane ("the substrate") to (*R*)-3-hydroxythiolane ("the product"), which method comprises contacting or incubating the substrate with a ketoreductase polypeptide of the disclosure under reaction conditions suitable for reducing or converting the substrate to the product. In some embodiments of this method, the substrate is reduced to the product with a stereomeric excess at least greater than about 65% or at least greater than wild-type.

[0022] In some embodiments of this method, the substrate is reduced to the product in greater than about 90% s.e., wherein the ketoreductase polypeptides comprise amino acid sequences corresponding to SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 86, 88, 90, 92, 94, 96, 100, 102, 104, 106, 108, 110, 112, 126, 128, 130, and 134.

[0023] In some embodiments of this method, the substrate is reduced to the product in greater than about 98% s.e., wherein the ketoreductase polypeptides comprise an amino acid sequences corresponding to SEQ ID NO: 6, 8, 10, 18, 20, 22, 24, 26, 28, 30, 34, 36, 38, 40, 42, 50, 52, 54, 58, 62, 66, 70, 72, 76, 78, 80, and 134.

[0024] In some embodiments of this method for reducing the substrate to the product, the substrate is reduced to the product at an improved rate of at least 1.5 times, 2 times, 3 times 4 times, 5 times, 10 times, 20 times or more as compared to the wild-type enzyme (SEQ ID NO:4), wherein the ketoreductase polypeptides comprise amino acid sequences corresponding to SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 64, 66, 68, 70, 74, 76, 78, 80, 86, 88, 90, 92, 104, 106, 110, 112, 124, 126, 130, and 134.

[0025] In some embodiments, the methods relate to use of the ketoreductase polypeptides in the synthesis of other compounds, such as drug compounds. In some embodiments, the methods relate to use of the ketoreductase polypeptides in the synthesis of antibiotic sulopenem (CP-70,429), having the following structural formula (III):



[0026] Thus, in some embodiments, in a method for the synthesis of the antibiotic of structural formula (III) (*i.e.*, CP-70,429), a step in the method can comprise contacting the substrate of formula (I) with any of the ketoreductases described herein, under reaction conditions suitable for converting or reducing the substrate of structural formula (I) to the product of structural formula (II). The compound of structural formula (II) in stereomeric excess of at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% or more can be used in the preparation of the compound of formula (III).

6. BRIEF DESCRIPTION OF THE DRAWINGS

[0027] FIG.1 illustrates the role of ketoreductases (KRED) in the conversion of the substrate compound of formula (I), 3-ketothiolane, to the corresponding chiral alcohol product of formula (II), (*R*)-3-hydroxythiolane. In this reaction, the substrate is reduced biocatalytically to the corresponding (*R*)-alcohol. This reduction uses a KRED of the disclosure and a co-factor such as NADPH. A glucose dehydrogenase (GDH) can be used to convert/recycle NADP⁺ to NADPH. Glucose is

converted to gluconic acid, which in turn is converted to its sodium salt (sodium gluconate) with the addition of sodium hydroxide. Example 9 provides a non-limiting method for conducting the reaction.

7. DETAILED DESCRIPTION

7.1 Definitions

[0028] As used herein, the following terms are intended to have the following meanings.

[0029] “Ketoreductase” and “KRED” are used interchangeably herein to refer to a polypeptide having an enzymatic capability of reducing a carbonyl group to its corresponding alcohol. More specifically, the ketoreductase polypeptides described herein are capable of stereoselectively reducing the compound of formula (I), *supra* to the corresponding product of formula (II), *supra*. The polypeptide typically utilizes a cofactor reduced nicotinamide adenine dinucleotide (NADH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH) as the reducing agent.

Ketoreductases as used herein include naturally occurring (wild type) ketoreductases as well as non-naturally occurring engineered polypeptides generated by human manipulation.

[0030] “Coding sequence” refers to that portion of a nucleic acid (*e.g.*, a gene) that encodes an amino acid sequence of a protein.

[0031] “Naturally-occurring” or “wild-type” refers to the form found in nature. For example, a naturally occurring or wild-type polypeptide or polynucleotide sequence is a sequence present in an organism that can be isolated from a source in nature and which has not been intentionally modified by human manipulation.

[0032] “Recombinant” when used with reference to, *e.g.*, a cell, nucleic acid, or polypeptide, refers to a material, or a material corresponding to the natural or native form of the material, that has been modified in a manner that would not otherwise exist in nature, or is identical thereto but produced or derived from synthetic materials and/or by manipulation using recombinant techniques. Non-limiting examples include, among others, recombinant cells expressing genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise expressed at a different level.

[0033] “Percentage of sequence identity” and “percentage homology” are used interchangeably herein to refer to comparisons among polynucleotides and polypeptides, and are determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) as compared to the reference sequence (which does not comprise additions or

deletions) for optimal alignment of the two sequences. The percentage may be calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Alternatively, the percentage may be calculated by determining the number of positions at which either the identical nucleic acid base or amino acid residue occurs in both sequences or a nucleic acid base or amino acid residue is aligned with a gap to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Those of skill in the art appreciate that there are many established algorithms available to align two sequences. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith and Waterman, 1981, *Adv. Appl. Math.* 2:482, by the homology alignment algorithm of Needleman and Wunsch, 1970, *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci. USA* 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the GCG Wisconsin Software Package), or by visual inspection (see generally, *Current Protocols in Molecular Biology*, F. M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1995 Supplement) (Ausubel)). Examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., 1990, *J. Mol. Biol.* 215: 403-410 and Altschul et al., 1977, *Nucleic Acids Res.* 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information website. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as, the neighborhood word score threshold (Altschul et al, supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T,

and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff, 1989, *Proc Natl Acad Sci USA* 89:10915). Exemplary determination of sequence alignment and % sequence identity can employ the BESTFIT or GAP programs in the GCG Wisconsin Software package (Accelrys, Madison WI), using default parameters provided.

[0034] “Reference sequence” refers to a defined sequence used as a basis for a sequence comparison. A reference sequence may be a subset of a larger sequence, for example, a segment of a full-length gene or polypeptide sequence. Generally, a reference sequence is at least 20 nucleotide or amino acid residues in length, at least 25 residues in length, at least 50 residues in length, or the full length of the nucleic acid or polypeptide. Since two polynucleotides or polypeptides may each (1) comprise a sequence (*i.e.*, a portion of the complete sequence) that is similar between the two sequences, and (2) may further comprise a sequence that is divergent between the two sequences, sequence comparisons between two (or more) polynucleotides or polypeptide are typically performed by comparing sequences of the two polynucleotides over a “comparison window” to identify and compare local regions of sequence similarity.

[0035] In some embodiments, a “reference sequence” can be based on a primary amino acid sequence, where the reference sequence is a sequence that can have one or more changes in the primary sequence. For instance, a reference sequence “based on SEQ ID NO:4 having at the residue corresponding to X145 a serine” refers to a reference sequence in which the corresponding residue at X145 in SEQ ID NO:4 has been changed to a serine.

[0036] “Comparison window” refers to a conceptual segment of at least about 20 contiguous nucleotide positions or amino acids residues wherein a sequence may be compared to a reference sequence of at least 20 contiguous nucleotides or amino acids and wherein the portion of the sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The comparison window can be longer than 20 contiguous residues, and includes, optionally 30, 40, 50, 100, or longer windows.

[0037] “Substantial identity” refers to a polynucleotide or polypeptide sequence that has at least 80 percent sequence identity, at least 85 percent identity and 89 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison window of at least 20 residue positions, frequently over a window of at least 30-50 residues, wherein

the percentage of sequence identity is calculated by comparing the reference sequence to a sequence that includes deletions or additions which total 20 percent or less of the reference sequence over the window of comparison. In specific embodiments applied to polypeptides, the term “substantial identity” means that two polypeptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 89 percent sequence identity, at least 95 percent sequence identity or more (*e.g.*, 99 percent sequence identity). Preferably, amino acid residue positions which are not identical differ by conservative amino acid substitutions.

[0038] “Corresponding to”, “reference to” or “relative to” when used in the context of the numbering of a given amino acid or polynucleotide sequence refers to the numbering of the residues of a specified reference sequence when the given amino acid or polynucleotide sequence is compared to the reference sequence. In other words, the residue number or residue position of a given polymer is designated with respect to the reference sequence rather than by the actual numerical position of the residue within the given amino acid or polynucleotide sequence. For example, a given amino acid sequence, such as that of an engineered ketoreductase, can be aligned to a reference sequence by introducing gaps to optimize residue matches between the two sequences. In these cases, although the gaps are present, the numbering of the residue in the given amino acid or polynucleotide sequence is made with respect to the reference sequence to which it has been aligned.

[0039] “Stereoselectivity” refers to the preferential formation in a chemical or enzymatic reaction of one stereoisomer over another. Stereoselectivity can be partial, where the formation of one stereoisomer is favored over the other, or it may be complete where only one stereoisomer is formed. When the stereoisomers are enantiomers, the stereoselectivity is referred to as enantioselectivity, the fraction (typically reported as a percentage) of one enantiomer in the sum of both. It is commonly alternatively reported in the art (typically as a percentage) as the enantiomeric excess (*e.e.*) calculated therefrom according to the formula $[\text{major enantiomer} - \text{minor enantiomer}] / [\text{major enantiomer} + \text{minor enantiomer}]$. This can also be referred to as stereomeric excess (*s.e.*). Where the stereoisomers are diastereoisomers, the stereoselectivity is referred to as diastereoselectivity, the fraction (typically reported as a percentage) of one diastereomer in a mixture of two diastereomers.

[0040] “Highly stereoselective” refers to a ketoreductase polypeptide that is capable of converting or reducing the substrate to the corresponding (*R*)-product with at least about 85% stereomeric excess.

[0041] “Improved enzyme property” refers to a ketoreductase polypeptide that exhibits an improvement in any enzyme property as compared to a reference ketoreductase. For the engineered ketoreductase polypeptides described herein, the comparison is generally made to the wild-type

ketoreductase enzyme, although in some embodiments, the reference ketoreductase can be another improved engineered ketoreductase. Enzyme properties for which improvement is desirable include, but are not limited to, enzymatic activity (which can be expressed in terms of percent conversion of the substrate), thermal stability, pH activity profile, cofactor requirements, refractoriness to inhibitors (e.g., product inhibition), stereospecificity, and stereoselectivity (including enantioselectivity).

[0042] “Increased enzymatic activity” refers to an improved property of the engineered ketoreductase polypeptides, which can be represented by an increase in specific activity (e.g., product produced/time/weight protein) or an increase in percent conversion of the substrate to the product (e.g., percent conversion of starting amount of substrate to product in a specified time period using a specified amount of KRED) as compared to the reference ketoreductase enzyme. Exemplary methods to determine enzyme activity are provided in the Examples. Any property relating to enzyme activity may be affected, including the classical enzyme properties of K_m , V_{max} or k_{cat} , changes of which can lead to increased enzymatic activity. Improvements in enzyme activity can be from about 1.5 times the enzymatic activity of the corresponding wild-type ketoreductase enzyme, to as much as 2 times, 5 times, 10 times, 20 times, 25 times, 50 times, 75 times, 100 times, or more enzymatic activity than the naturally occurring ketoreductase or another engineered ketoreductase from which the ketoreductase polypeptides were derived. In specific embodiments, the engineered ketoreductase enzyme exhibits improved enzymatic activity in the range of 1.5 to 50 times or 1.5 to 100 times greater than that of the parent ketoreductase enzyme. It is understood by the skilled artisan that the activity of any enzyme is diffusion limited such that the catalytic turnover rate cannot exceed the diffusion rate of the substrate, including any required cofactors. The theoretical maximum of the diffusion limit, or k_{cat}/K_m , is generally about 10^8 to 10^9 ($M^{-1} s^{-1}$). Hence, any improvements in the enzyme activity of the ketoreductase will have an upper limit related to the diffusion rate of the substrates acted on by the ketoreductase enzyme. Ketoreductase activity can be measured by any one of standard assays used for measuring ketoreductase, such as a decrease in absorbance or fluorescence of NADPH (see Example 6) due to its oxidation with the concomitant reduction of a ketone or aldehyde to an alcohol, or by product produced in a coupled assay. Comparisons of enzyme activities are made using a defined preparation of enzyme, a defined assay under a set condition, and one or more defined substrates, as further described in detail herein. Generally, when lysates are compared, the numbers of cells and the amount of protein assayed are determined as well as use of identical expression systems and identical host cells to minimize variations in amount of enzyme produced by the host cells and present in the lysates.

[0043] “Conversion” refers to the enzymatic reduction of the substrate to the corresponding product. “Percent conversion” refers to the percent of the substrate that is reduced to the product within a

period of time under specified conditions. Thus, the “enzymatic activity” or “activity” of a ketoreductase polypeptide can be expressed as “percent conversion” of the substrate to the product.

[0044] “Thermostable” refers to a ketoreductase polypeptide that maintains similar activity (more than 60% to 80% for example) after exposure to elevated temperatures (*e.g.*, 40-80°C) for a period of time (*e.g.*, 0.5-24 hrs) compared to the untreated enzyme.

[0045] “Solvent stable” refers to a ketoreductase polypeptide that maintains similar activity (more than *e.g.* 60% to 80%) after exposure to varying concentrations (*e.g.*, 5-99%) of solvent (*e.g.*, isopropylalcohol, tetrahydrofuran, 2-methyltetrahydrofuran, acetone, toluene, butylacetate, methyl tert-butylether, etc.) for a period of time (*e.g.*, 0.5-24 hrs) compared to the untreated enzyme.

[0046] “pH stable” refers to a ketoreductase polypeptide that maintains similar activity (more than *e.g.*, 60% to 80%) after exposure to high or low pH (*e.g.*, 4.5-6 or 8 to 12) for a period of time (*e.g.*, 0.5-24 hrs) compared to the untreated enzyme.

[0047] “Thermo- and solvent stable” refers to a ketoreductase polypeptide that are both thermostable and solvent stable.

[0048] “Derived from” as used herein in the context of engineered ketoreductase enzymes, identifies the originating ketoreductase enzyme, and/or the gene encoding such ketoreductase enzyme, upon which the engineering was based. For example, the engineered ketoreductase enzyme of SEQ ID NO: 134 was obtained by artificially evolving, over multiple generations the gene encoding the *L. kefir* ketoreductase enzyme of SEQ ID NO:4. Thus, this engineered ketoreductase enzyme is “derived from” the wild-type ketoreductase of SEQ ID NO.: 4.

[0049] “Hydrophilic Amino Acid or Residue” refers to an amino acid or residue having a side chain exhibiting a hydrophobicity of less than zero according to the normalized consensus hydrophobicity scale of Eisenberg *et al.*, 1984, *J. Mol. Biol.* 179:125-142. Genetically encoded hydrophilic amino acids include L-Thr (T), L-Ser (S), L-His (H), L-Glu (E), L-Asn (N), L-Gln (Q), L-Asp (D), L-Lys (K) and L-Arg (R).

[0050] “Acidic Amino Acid or Residue” refers to a hydrophilic amino acid or residue having a side chain exhibiting a pK value of less than about 6 when the amino acid is included in a peptide or polypeptide. Acidic amino acids typically have negatively charged side chains at physiological pH due to loss of a hydrogen ion. Genetically encoded acidic amino acids include L-Glu (E) and L-Asp (D).

[0051] “Basic Amino Acid or Residue” refers to a hydrophilic amino acid or residue having a side chain exhibiting a pK value of greater than about 6 when the amino acid is included in a peptide or

polypeptide. Basic amino acids typically have positively charged side chains at physiological pH due to association with hydronium ion. Genetically encoded basic amino acids include L-Arg (R) and L-Lys (K).

[0052] “Polar Amino Acid or Residue” refers to a hydrophilic amino acid or residue having a side chain that is uncharged at physiological pH, but which has at least one bond in which the pair of electrons shared in common by two atoms is held more closely by one of the atoms. Genetically encoded polar amino acids include L-Asn (N), L-Gln (Q), L-Ser (S) and L-Thr (T).

[0053] “Hydrophobic Amino Acid or Residue” refers to an amino acid or residue having a side chain exhibiting a hydrophobicity of greater than zero according to the normalized consensus hydrophobicity scale of Eisenberg *et al.*, 1984, *J. Mol. Biol.* 179:125-142. Genetically encoded hydrophobic amino acids include L-Pro (P), L-Ile (I), L-Phe (F), L-Val (V), L-Leu (L), L-Trp (W), L-Met (M), L-Ala (A) and L-Tyr (Y).

[0054] “Aromatic Amino Acid or Residue” refers to a hydrophilic or hydrophobic amino acid or residue having a side chain that includes at least one aromatic or heteroaromatic ring. Genetically encoded aromatic amino acids include L-Phe (F), L-Tyr (Y) and L-Trp (W). Although owing to the pKa of its heteroaromatic nitrogen atom L-His (H) it is sometimes classified as a basic residue, or as an aromatic residue as its side chain includes a heteroaromatic ring, herein histidine is classified as a hydrophilic residue or as a “constrained residue” (see below).

[0055] “Constrained amino acid or residue” refers to an amino acid or residue that has a constrained geometry. Herein, constrained residues include L-pro (P) and L-his (H). Histidine has a constrained geometry because it has a relatively small imidazole ring. Proline has a constrained geometry because it also has a five membered ring.

[0056] “Non-polar Amino Acid or Residue” refers to a hydrophobic amino acid or residue having a side chain that is uncharged at physiological pH and which has bonds in which the pair of electrons shared in common by two atoms is generally held equally by each of the two atoms (*i.e.*, the side chain is not polar). Genetically encoded non-polar amino acids include L-Gly (G), L-Leu (L), L-Val (V), L-Ile (I), L-Met (M) and L-Ala (A).

[0057] “Aliphatic Amino Acid or Residue” refers to a hydrophobic amino acid or residue having an aliphatic hydrocarbon side chain. Genetically encoded aliphatic amino acids include L-Ala (A), L-Val (V), L-Leu (L) and L-Ile (I).

[0058] “Cysteine”. The amino acid L-Cys (C) is unusual in that it can form disulfide bridges with other L-Cys (C) amino acids or other sulfanyl- or sulfhydryl-containing amino acids. The “cysteine-

like residues" include cysteine and other amino acids that contain sulfhydryl moieties that are available for formation of disulfide bridges. The ability of L-Cys (C) (and other amino acids with -SH containing side chains) to exist in a peptide in either the reduced free -SH or oxidized disulfide-bridged form affects whether L-Cys (C) contributes net hydrophobic or hydrophilic character to a peptide. While L-Cys (C) exhibits a hydrophobicity of 0.29 according to the normalized consensus scale of Eisenberg (Eisenberg *et al.*, 1984, *supra*), it is to be understood that for purposes of the present disclosure L-Cys (C) is categorized into its own unique group.

[0059] "Small Amino Acid or Residue" refers to an amino acid or residue having a side chain that is composed of a total three or fewer carbon and/or heteroatoms (excluding the α -carbon and hydrogens). The small amino acids or residues may be further categorized as aliphatic, non-polar, polar or acidic small amino acids or residues, in accordance with the above definitions. Genetically-encoded small amino acids include L-Ala (A), L-Val (V), L-Cys (C), L-Asn (N), L-Ser (S), L-Thr (T) and L-Asp (D).

[0060] "Hydroxyl-containing Amino Acid or Residue" refers to an amino acid containing a hydroxyl (-OH) moiety. Genetically-encoded hydroxyl-containing amino acids include L-Ser (S) L-Thr (T) and L-Tyr (Y).

[0061] "Conservative" amino acid substitutions or mutations refer to the interchangeability of residues having similar side chains, and thus typically involves substitution of the amino acid in the polypeptide with amino acids within the same or similar defined class of amino acids. However, as used herein, in some embodiments, conservative mutations do not include substitutions from a hydrophilic to hydrophilic, hydrophobic to hydrophobic, hydroxyl-containing to hydroxyl-containing, or small to small residue, if the conservative mutation can instead be a substitution from an aliphatic to an aliphatic, non-polar to non-polar, polar to polar, acidic to acidic, basic to basic, aromatic to aromatic, or constrained to constrained residue. Further, as used herein, A, V, L, or I can be conservatively mutated to either another aliphatic residue or to another non-polar residue. The table below shows exemplary conservative substitutions.

Residue	Possible Conservative Mutations
A, L, V, I	Other aliphatic (A, L, V, I) Other non-polar (A, L, V, I, G, M)
G, M	Other non-polar (A, L, V, I, G, M)
D, E	Other acidic (D, E)
K, R	Other basic (K, R)
P, H	Other constrained (P, H)
N, Q, S, T	Other polar
Y, W, F	Other aromatic (Y, W, F)
C	None

[0062] “Non-conservative substitution” refers to substitution or mutation of an amino acid in the polypeptide with an amino acid with significantly differing side chain properties. Non-conservative substitutions may use amino acids between, rather than within, the defined groups listed above. In one embodiment, a non-conservative mutation affects (a) the structure of the peptide backbone in the area of the substitution (*e.g.*, proline for glycine) (b) the charge or hydrophobicity, or (c) the bulk of the side chain.

[0063] “Deletion” refers to modification to the polypeptide by removal of one or more amino acids from the reference polypeptide. Deletions in an amino acid sequence can comprise removal of 1 or more amino acids, 2 or more amino acids, 3 or more amino acids, 4 or more amino acid, 5 or more amino acids, 6 or more amino acids, 8 or more amino acids, 10 or more amino acids, 15 or more amino acids, or 20 or more amino acids, up to 10% of the total number of amino acids, up to 15% of the total number of amino acids, or up to 20% of the total number of amino acids making up the reference enzyme while retaining enzymatic activity and/or retaining the improved properties of an engineered ketoreductase enzyme. Deletions can be directed to the internal portions and/or terminal portions of the polypeptide. In various embodiments, the deletion can comprise a continuous segment or can be discontinuous.

[0064] “Insertion” refers to modification to the polypeptide by addition of one or more amino acids as compared to a reference polypeptide. In some embodiments, the improved engineered ketoreductase enzymes comprise insertions of one or more amino acids to the naturally occurring ketoreductase polypeptide as well as insertions of one or more amino acids to other improved ketoreductase polypeptides. Insertions can be in the internal portions of the polypeptide, or to the carboxy or amino terminus. Insertions as used herein include fusion proteins as is known in the art. The insertion can be a contiguous segment of amino acids or separated by one or more of the amino acids in the naturally occurring polypeptide.

[0065] “Fragment” refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the sequence. Fragments can be at least 14 amino acids long, at least 20 amino acids long, at least 50 amino acids long or longer, and up to 70%, 80%, 90%, 95%, 98%, and 99% of the full-length ketoreductase polypeptide. In some embodiments, the full length ketoreductase can be an full length engineered or wild type ketoreductase (*e.g.*, SEQ ID NO:2 and 4).

[0066] “Isolated polypeptide” refers to a polypeptide which is substantially separated from other contaminants that naturally accompany it, *e.g.*, protein, lipids, and polynucleotides. The term

embraces polypeptides which have been removed or purified from their naturally-occurring environment or expression system (e.g., host cell or *in vitro* synthesis). The improved ketoreductase enzymes may be present within a cell, present in the cellular medium, or prepared in various forms, such as lysates or isolated preparations. As such, in some embodiments, the improved ketoreductase enzyme can be an isolated polypeptide.

[0067] “Substantially pure polypeptide” refers to a composition in which the polypeptide species is the predominant species present (*i.e.*, on a molar or weight basis it is more abundant than any other individual macromolecular species in the composition), and is generally a substantially purified composition when the object species comprises at least about 50 percent of the macromolecular species present by mole or % weight. Generally, a substantially pure ketoreductase composition will comprise about 60 % or more, about 70% or more, about 80% or more, about 90% or more, about 95% or more, and about 98% or more of all macromolecular species by mole or % weight present in the composition. In some embodiments, the object species is purified to essential homogeneity (*i.e.*, contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species. Solvent species, small molecules (<500 Daltons), and elemental ion species are not considered macromolecular species. In some embodiments, the isolated improved ketoreductases polypeptide is a substantially pure polypeptide composition.

[0068] “Stringent hybridization” is used herein to refer to conditions under which nucleic acid hybrids are stable. As known to those of skill in the art, the stability of hybrids is reflected in the melting temperature (T_m) of the hybrids. In general, the stability of a hybrid is a function of ion strength, temperature, G/C content, and the presence of chaotropic agents. The T_m values for polynucleotides can be calculated using known methods for predicting melting temperatures (see, e.g., Baldino et al., 1989, *Methods Enzymology* 168:761-777; Bolton et al., 1962, *Proc. Natl. Acad. Sci. USA* 48:1390; Bresslauer et al., 1986, *Proc. Natl. Acad. Sci. USA* 83:8893-8897; Freier et al., 1986, *Proc. Natl. Acad. Sci. USA* 83:9373-9377; Kierzek et al., *Biochemistry* 25:7840-7846; Rychlik et al., 1990, *Nucleic Acids Res* 18:6409-6412 (erratum, 1991, *Nucleic Acids Res* 19:698); Sambrook et al., *supra*; Suggs et al., 1981, In *Developmental Biology Using Purified Genes* (Brown et al., eds.), pp. 683-693, Academic Press; and Wetmur, 1991, *Crit Rev Biochem Mol Biol* 26:227-259; all publications incorporate herein by reference). In some embodiments, the polynucleotide encodes the polypeptide disclosed herein and hybridizes under defined conditions, such as moderately stringent or highly stringent conditions, to the complement of a sequence encoding an engineered ketoreductase enzyme of the present disclosure.

[0069] “Hybridization stringency” relates to hybridization conditions, such as washing conditions, in the hybridization of nucleic acids. Generally, hybridization reactions are performed under conditions of lower stringency, followed by washes of varying but higher stringency. The term “moderately stringent hybridization” refers to conditions that permit target-DNA to bind a complementary nucleic acid that has about 60% identity, preferably about 75% identity, about 85% identity to the target DNA, with greater than about 90% identity to target-polynucleotide. Exemplary moderately stringent conditions are conditions equivalent to hybridization in 50% formamide, 5× Denhart's solution, 5×SSPE, 0.2% SDS at 42°C., followed by washing in 0.2×SSPE, 0.2% SDS, at 42°C. “High stringency hybridization” refers generally to conditions that are about 10°C or less from the thermal melting temperature T_m as determined under the solution condition for a defined polynucleotide sequence. In some embodiments, a high stringency condition refers to conditions that permit hybridization of only those nucleic acid sequences that form stable hybrids in 0.018M NaCl at 65°C. (i.e., if a hybrid is not stable in 0.018M NaCl at 65°C, it will not be stable under high stringency conditions, as contemplated herein). High stringency conditions can be provided, for example, by hybridization in conditions equivalent to 50% formamide, 5× Denhart's solution, 5×SSPE, 0.2% SDS at 42°C, followed by washing in 0.1×SSPE, and 0.1% SDS at 65°C. Another high stringency condition is hybridizing in conditions equivalent to hybridizing in 5X SSC containing 0.1% (w:v) SDS at 65°C and washing in 0.1x SSC containing 0.1% SDS at 65°C. Other high stringency hybridization conditions, as well as moderately stringent conditions, are described in the references cited above.

[0070] “Heterologous” polynucleotide refers to any polynucleotide that is introduced into a host cell by laboratory techniques, and includes polynucleotides that are removed from a host cell, subjected to laboratory manipulation, and then reintroduced into a host cell.

[0071] “Codon optimized” refers to changes in the codons of the polynucleotide encoding a protein to those preferentially used in a particular organism such that the encoded protein is efficiently expressed in the organism of interest. Although the genetic code is degenerate in that most amino acids are represented by several codons, called “synonyms” or “synonymous” codons, it is well known that codon usage by particular organisms is nonrandom and biased towards particular codon triplets. This codon usage bias may be higher in reference to a given gene, genes of common function or ancestral origin, highly expressed proteins versus low copy number proteins, and the aggregate protein coding regions of an organism's genome. In some embodiments, the polynucleotides encoding the ketoreductases enzymes may be codon optimized for optimal production in the host organism selected for expression.

[0072] “Preferred, optimal, high codon usage bias codons” refers interchangeably to codons that are used at higher frequency in the protein coding regions than other codons that code for the same amino acid. The preferred codons may be determined in relation to codon usage in a single gene, a set of genes of common function or origin, highly expressed genes, the codon frequency in the aggregate protein coding regions of the whole organism, codon frequency in the aggregate protein coding regions of related organisms, or combinations thereof. Codons whose frequency increases with the level of gene expression are typically optimal codons for expression. A variety of methods are known for determining the codon frequency (*e.g.*, codon usage, relative synonymous codon usage) and codon preference in specific organisms, including multivariate analysis, for example, using cluster analysis or correspondence analysis, and the effective number of codons used in a gene (see GCG CodonPreference, Genetics Computer Group Wisconsin Package; CodonW, John Peden, University of Nottingham; McInerney, J. O, 1998, *Bioinformatics* 14:372-73; Stenico et al., 1994, *Nucleic Acids Res.* 22:2437-46; Wright, F., 1990, *Gene* 87:23-29). Codon usage tables are available for a growing list of organisms (see for example, Wada et al., 1992, *Nucleic Acids Res.* 20:2111-2118; Nakamura et al., 2000, *Nucl. Acids Res.* 28:292; Duret, et al., *supra*; Henaut and Danchin, “*Escherichia coli* and *Salmonella*,” 1996, Neidhardt, et al. Eds., ASM Press, Washington D.C., p. 2047-2066. The data source for obtaining codon usage may rely on any available nucleotide sequence capable of coding for a protein. These data sets include nucleic acid sequences actually known to encode expressed proteins (*e.g.*, complete protein coding sequences-CDS), expressed sequence tags (ESTS), or predicted coding regions of genomic sequences (see for example, Mount, D., *Bioinformatics: Sequence and Genome Analysis*, Chapter 8, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001; Uberbacher, E. C., 1996, *Methods Enzymol.* 266:259-281; Tiwari et al., 1997, *Comput. Appl. Biosci.* 13:263-270).

[0073] “Control sequence” is defined herein to include all components, which are necessary or advantageous for the expression of a polynucleotide or polypeptide of interest. Each control sequence may be native or foreign to the nucleic acid sequence encoding a polypeptide. Such control sequences include, but are not limited to, a leader sequence, polyadenylation sequence, propeptide sequence, promoter, signal peptide sequence, and transcription terminator. At a minimum, the control sequences include a promoter, and transcriptional and translational stop signals. The control sequences may be provided with linkers for the purpose of introducing specific restriction sites facilitating ligation of the control sequences with the coding region of the nucleic acid sequence encoding a polypeptide.

[0074] “Operably linked” is defined herein as a configuration in which a control sequence is appropriately placed in a functional relationship (*i.e.*, at a position relative to) with a polynucleotide or

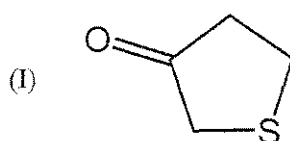
polypeptide of interest, such as the coding sequence in the DNA sequence, such that the control sequence directs or regulates the expression of a polynucleotide and/or polypeptide.

[0075] “Promoter sequence” is a nucleic acid sequence that is recognized by a host cell for expression of a polynucleotide of interest, such as a coding region. Generally, the promoter sequence contains transcriptional control sequences, which mediate the expression of a polynucleotide of interest. The promoter may be any nucleic acid sequence which shows transcriptional activity in the host cell of choice, including mutant, truncated, and hybrid promoters, and may be obtained from genes encoding extracellular or intracellular polypeptides either homologous or heterologous to the host cell.

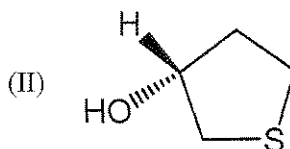
[0076] “Cofactor regeneration system” refers to a set of reactants that participate in a reaction that reduces the oxidized form of the cofactor (*e.g.*, NADP⁺ to NADPH). Cofactors oxidized by the ketoreductase-catalyzed reduction of the keto substrate are regenerated in reduced form by the cofactor regeneration system. Cofactor regeneration systems comprise a stoichiometric reductant that is a source of reducing hydrogen equivalents and is capable of reducing the oxidized form of the cofactor. The cofactor regeneration system may further comprise a catalyst, for example an enzyme catalyst, that catalyzes the reduction of the oxidized form of the cofactor by the reductant. Cofactor regeneration systems to regenerate NADH or NADPH from NAD⁺ or NADP⁺, respectively, are known in the art and may be used in the methods described herein.

7.2 Ketoreductase Enzymes

[0077] The present disclosure provides engineered ketoreductase (“KRED”) enzymes that are capable of stereoselectively reducing or converting 3-ketothiolane (“the substrate”) of structural formula (I):



to (*R*)-3-hydroxythiolane (“the product”) of structural formula (II):



and having an improved property when compared with the naturally-occurring, wild-type KRED enzyme obtained from *L. kefir* (SEQ ID NO:4), *L. brevis* (SEQ ID NO:2) or *L. minor* (SEQ ID

NO:142), or when compared with other engineered ketoreductase enzymes. Enzyme properties for which improvement is desirable include, but are not limited to, enzymatic activity, thermal stability, pH activity profile, cofactor requirements, refractoriness to inhibitors (*e.g.*, product inhibition), stereospecificity, stereoselectivity, and solvent stability. The improvements can relate to a single enzyme property, such as enzymatic activity, or a combination of different enzyme properties, such as enzymatic activity and stereoselectivity.

[0078] The present disclosure provides engineered ketoreductase (“KRED”) enzymes that are capable of stereoselectively reducing a defined keto substrate to its corresponding alcohol product and having an improved property when compared with the naturally-occurring, wild-type KRED enzyme obtained from *L. kefir* (SEQ ID NO:2) or *L. brevis* (SEQ ID NO:4) or *L. minor* (SEQ ID NO:142), or when compared with other engineered ketoreductase enzymes. The ketoreductase polypeptides of the disclosure have the requirement that the residue corresponding to position 145 of SEQ ID NO:2 or 4 or 142 is not an acidic residue. In some embodiments, the ketoreductase polypeptides have, as compared to the *L. kefir* or *L. brevis* or *L. minor* KRED sequences of SEQ ID NO:2 or 4 or 142, at the residue corresponding to position 145 a polar residue. In some embodiments, the ketoreductase polypeptides have, as compared to the *L. kefir* or *L. brevis* or *L. minor* KRED sequences of SEQ ID NO:2 or 4 or 142, at the residue corresponding to position 145 a serine.

[0079] In some embodiments, as noted above, the engineered ketoreductase with improved enzyme activity is described with reference to *L. kefir* ketoreductase of SEQ ID NO:4, *L. brevis* ketoreductase of SEQ ID NO:2, *L. minor* of SEQ ID NO:142, or an engineered ketoreductase. The amino acid residue position is determined in these ketoreductases beginning from the initiating methionine (M) residue (*i.e.*, M represents residue position 1), although it will be understood by the skilled artisan that this initiating methionine residue may be removed by biological processing machinery, such as in a host cell or in vitro translation system, to generate a mature protein lacking the initiating methionine residue. The amino acid residue position at which a particular amino acid or amino acid change is present in an amino acid sequence is sometimes describe herein in terms “X_n”, or “position n”, where n refers to the residue position. Where the amino acid residues at the same residue position differ between the ketoreductases, the different residues are denoted by an “/” with the arrangement being, for example, “kefir residue/brevis residue/minor.” In some embodiments, a substitution mutation, which is a replacement of an amino acid residue in a corresponding residue of a reference sequence, for example the wildtype ketoreductase of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:142 with a different amino acid residue is denoted by the symbol “→”.

[0080] Herein, mutations are sometimes described as a mutation “to a” type of amino acid. For example, residue X7 can be mutated glycine “to a” serine residue. But the use of the phrase “to a” does not exclude mutations from one amino acid of a class to another amino acid of the same class. For example, residue X7 can be mutated from a glycine to an arginine.

[0081] The polynucleotide sequence encoding the naturally occurring ketoreductase (also referred to as “ADH” or “alcohol dehydrogenase”) of *L. kefir*, *L. brevis*, or of *L. minor*, and thus the corresponding amino acid sequences, are available as Genbank accession no. AAP94029 GI:33112056 or SEQ ID NO:3 for *L. kefir*; Genbank accession no. CAD66648 GI:28400789 or SEQ ID NO:1 for *L. brevis*; and SEQ ID NO:141 for *L. minor*).

[0082] In some embodiments, the ketoreductase polypeptides herein can have a number of modifications to the reference sequence (*e.g.*, naturally occurring polypeptide or an engineered polypeptide) to result in an improved ketoreductase property. In such embodiments, the number of modifications to the amino acid sequence can comprise one or more amino acids, 2 or more amino acids, 3 or more amino acids, 4 or more amino acids, 5 or more amino acids, 6 or more amino acids, 8 or more amino acids, 10 or more amino acids, 15 or more amino acids, or 20 or more amino acids, up to 10% of the total number of amino acids, up to 10% of the total number of amino acids, up to 15% of the total number of amino acids, up to 20% of the total number of amino acids, or up to 30% of the total number of amino acids of the reference polypeptide sequence. In some embodiments, the number of modifications to the naturally occurring polypeptide or an engineered polypeptide that produces an improved ketoreductase property may comprise from about 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-26, 1-30, 1-35 or about 1-40 residue modifications of the reference sequence. In some embodiments, the number of modifications can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 amino acid residues. The modifications can comprise insertions, deletions, substitutions, or combinations thereof.

[0083] In some embodiments, the modifications comprise amino acid substitutions to the reference sequence. Substitutions that can produce an improved ketoreductase property may be at one or more amino acids, 2 or more amino acids, 3 or more amino acids, 4 or more amino acids, 5 or more amino acids, 6 or more amino acids, 8 or more amino acids, 10 or more amino acids, 15 or more amino acids, or 20 or more amino acids, up to 10% of the total number of amino acids, up to 10% of the total number of amino acids, up to 20% of the total number of amino acids, or up to 30% of the total number of amino acids of the reference enzyme sequence. In some embodiments, the number of substitutions to the naturally occurring polypeptide or an engineered polypeptide that produces an improved ketoreductase property can comprise from about 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-26, 1-30, 1-35 or about 1-40 amino acid

substitutions of the reference sequence. In some embodiments, the number of substitutions can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 amino acid residues.

[0084] In some embodiments, the improved property (as compared to wild-type or another engineered polypeptide) of the ketoreductase polypeptide is with respect to an increase of its stereoselectivity for reducing or converting 3-ketothiolane substrate to (*R*)-3-hydroxythiolane. In some embodiments, the ketoreductase polypeptide is capable of stereoselectively reducing the substrate to the product with a percent stereomeric excess of at least about 65%, 70%, 75%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 99.99%.

[0085] In some embodiments, the ketoreductase polypeptide is capable of stereoselectively reducing the substrate to the product with a percent stereomeric excess (s.e.) that is improved as compared to the wild-type *L. kefir* or *L. brevis* or *L. minor* KRED (SEQ ID NO:4 or 2 or 142). In some embodiments, the ketoreductase polypeptide is capable of stereoselectively reducing the substrate to the product with a percent stereomeric excess that is at least about 70%.

[0086] In some embodiments, the ketoreductase polypeptides of the disclosure are highly stereoselective in that the ketoreductases can reduce the substrate to the product in greater than about 90% stereomeric excess (s.e.). Exemplary ketoreductase polypeptides with such high stereoselectivity include, but are not limited to, the polypeptides comprising the amino acid sequences corresponding to SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 86, 88, 90, 92, 94, 96, 100, 102, 104, 106, 108, 110, 112, 126, 128, 130, and 134.

[0087] In some embodiments, the ketoreductase polypeptides of the disclosure are improved as compared to wild-type (SEQ ID NO:4) with respect to their rate of enzymatic activity, *i.e.*, their rate or ability of converting the substrate to the product. Exemplary polypeptides that are capable of converting the substrate to the product at a rate that is improved over wild-type, include but are not limited to, polypeptides comprising the amino acid sequences corresponding to SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 64, 66, 68, 70, 74, 76, 78, 80, 86, 88, 90, 92, 104, 106, 110, 112, 124, 126, 130, and 134.

[0088] In some embodiments, the ketoreductase polypeptides of the disclosure are highly stereoselective and can reduce the substrate to the product in greater than about 98% s.e. Exemplary polypeptides with such stereoselectivity include, but are not limited to, the polypeptides comprising the amino acid sequences corresponding to SEQ ID NO: 6, 8, 10, 18, 20, 22, 24, 26, 28, 30, 34, 36, 38, 40, 42, 50, 52, 54, 58, 62, 66, 70, 72, 76, 78, 80, and 134.

[0089] In some embodiments, the ketoreductase polypeptides of the disclosure are improved as compared to wild-type with respect to their thermostability, as determined by an increase in the rate of enzymatic activity as compared to wild-type under high temperatures. Exemplary ketoreductase polypeptides with improved stability, include but are not limited to, polypeptides comprising amino acid sequences corresponding to SEQ ID NO: 6, 8, 12, 14, 22, 24, 26, 32, 34, 36, 38, 42, 44, 46, 50, 52, 56, 58, 60, 64, 66, 68, 70, 74, 76, 78, 80, 82, 86, 88, 90, 92, 104, 106, 110, 112, 124, and 134.

[0090] In some embodiments, the ketoreductase polypeptides of the disclosure have improved activity and stability over wild-type, and can reduce the substrate to the product in greater than about 95% s.e. Exemplary polypeptides with such capabilities include, but are not limited to, polypeptides comprising amino acid sequences corresponding to SEQ ID NO: 6, 8, 12, 14, 22, 24, 26, 30, 32, 38, 42, 44, 46, 50, 52, 56, 58, 60, 64, 66, 68, 70, 74, 76, 78, 80, 82, 86, 88, 90, 92, 104, 106, 110, 112, and 134.

[0091] In some embodiments, the ketoreductases of the disclosure are capable of reducing or converting at least 100 g/L of ketothiolane to the corresponding product in less than 24 hrs (e.g., about 20-24 hours) at room temperature with about 0.8-1.0 g/L of the ketoreductase polypeptide. Exemplary polypeptides with such capabilities, include, but are not limited to polypeptides comprising amino acid sequences corresponding to SEQ ID NO: 6, 8, 10, 12, 14, 18, 20, 22, 24, 26, 30, 32, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 64, 66, 68, 70, 74, 76, 78, 80, 82, 86, 88, 90, 92, 104, 106, 110, 112, 126, 130, and 134.

[0092] In some embodiments, the ketoreductases of the disclosure are capable of reducing or converting at least 100 g/L of ketothiolane to product in less than 20 hrs (e.g., 12-20 hrs) at room temperature with about 0.8-1.0 g/L of the ketoreductase polypeptide. Exemplary polypeptides with such capabilities, include, but are not limited to SEQ ID NO: 26, 44, 68, and 104.

[0093] Table 2 below provides exemplary ketoreductases. All sequences below are derived from the wild-type *L. kefir* ketoreductase sequences (SEQ ID NO: 3 and 4) unless otherwise specified. Each of the polypeptide sequences in Table 2 are encoded by the nucleotide sequence having the SEQ ID NO that is one less than the number in the Table, i.e., the polypeptide of SEQ ID NO:4 is encoded by the nucleotide sequence of SEQ ID NO:3.

[0094] Table 2: List of Sequences and Properties

SEQ ID NO	Mutations as Compared to SEQ ID NO:4	Stereoselectivity	Activity	Stability
4	None	0	0	0

SEQ ID NO	Mutations as Compared to SEQ ID NO:4	Stereoselectivity	Activity	Stability
120	N157S	0	0	0
116	E145D	0	0	0
122	G53V N157S	0	0	0
114	N157S V228A	+	0	0
118	A94T I126V N157S	+	0	0
132	K8R I23F A94T V95A R108H N157S	+	0	0
84	I11T A94T N157S	+	0	0
98	E145S	++	0	0
124	A41V K49R Q127R F147L E200K I217F	0	++	+
102	E145S N157T	+++	0	0
90	G117S E145S	+++	+	+
16	G7S E145S I223V	+++	+	0
100	E145S I217F	+++	0	0
108	E145S M214V	+++	0	0
128	T16A I57V E145S	+++	0	0
96	E145S M214T	+++	0	0
130	T16A E145S	+++	+	0
126	I19V E145S	+++	+	0
104	K72R G117S E145S N157T I223V	+++	++	+
88	G117S E145S N157T	+++	+	+
106	K49R R108H G117S E145S N157T I223V	+++	+	+
14	G7S R108H G117S E145S N157T	+++	+	+
86	R108H G117S E145S N157T K192R I223V	+++	+	+
44	G7S R108H G117S E145S N157T I223V	+++	++	+
92	G117S E145S N157T K192R	+++	+	+
112	E45G R108H G117S E145S N157T K192R D198G I223V	+++	+	+
94	G117S E145S T152M N157T	+++	0	-
12	G7S G117S E145S N157T	+++	+	+
56	G7S I57V K97E R108H G117S E145S N157T I223V	+++	+	+

SEQ ID NO	Mutations as Compared to SEQ ID NO:4	Stereoselectivity	Activity	Stability
110	E45G R108H G117S E145S N157T I223V	+++	+	+
36	G7S A94T R108H G117S E145S N157T I223V	++++	0	+
46	G7S R108H G117S E145S N157T K192E I223V	+++	+	+
68	G7S K49R L111M G117S E145S N157T D173G I223V	+++	++	+
48	G7S R108H G117S E145S N157T K192E I217F I223V	+++	+	-
74	G7S T16G R108H G117S E145S N157T I223V	+++	+	+
82	G7H R108H G117S E145S N157T I223V	+++	+	+
42	G7S A94T K97L R108H G117S E145S N157T I223V I226L	++++	+	+
24	G7S A94T S96P R108H G117S E145S N157T I223V	++++	+	+
34	G7S A94T S96A R108H G117S E145S N157T I223V	++++	0	+
38	G7S A94T R108H G117S E145S N157T M206Q I223V	++++	+	+
32	G7S A94T S96E R108H G117S E145S N157T I223V	+++	+	+
8	G7N A94T E106D R108H G117S E145S N157T I223V	++++	+	+
134	D3Y G7S A94T R108H G117S E145S N157T I223V	++++	+	+
6	G7S A94T R108H G117S E145S N157T Q208H I223V	++++	+	+
26	G7S A94T S96P R108H G117S E145S N157T M206Q I223V	+++++	++	+
72	G7S T16G A94T S96P R108H G117S E145S N157T M206Q I223V	+++++	0	-
18	G7S A94T S96P R108H G117S E145S F147L N157T P194L I223V	++++	+	0
20	G7S A64T A94T S96P R108H G117S E145S N157T I223V	++++	+	0

SEQ ID NO	Mutations as Compared to SEQ ID NO:4	Stereoselectivity	Activity	Stability
54	G7S A94T S96P R108H G117S E145S N157T I217F I223V	++++	+	0
10	G7S A94T R108H G117S E145S N157T K177R M206Q Q208R I223V	++++	+	0
40	G7S A94T K97R R108H G117S E145S N157T M206Q I223V	++++	+	0
52	G7S R108H G117S E145S N157T M206Q I223V	++++	+	+
50	G7S S96P R108H G117S E145S N157T M206Q I223V	++++	+	+
76	G7S T16G S96P R108H G117S E145S N157T M206Q I223V	++++	+	+
80	G7S T16V A94T S96P R108H G117S E145S N157T M206Q I223V	+++++	+	+
78	G7S T16G A94V S96P R108H G117S E145S N157T M206Q I223V	++++	+	+
30	G7S A94T S96P R108H G117S E145S N157T P194G M206Q I223V	+++++	+	+
28	G7S A94T S96P R108H G117S E145S N157T P194D M206Q I223V	+++++	0	0
22	G7S A94T S96P R108H G117S E145S N157T P194N M206Q I223V	+++++	+	+
60	G7S K49R A94T L111M G117S E145S N157T D173G I223V	+++	+	+
64	G7S K49R S96P L111M G117S E145S N157T D173G I223V	+++	+	+
70	G7S K49R L111M G117S E145S N157T D173G M206Q I223V	++++	+	+
62	G7S K49R A94T L111M G117S E145S N157T D173G M206Q I223V	++++	0	0
66	G7S K49R S96P L111M G117S E145S N157T D173G M206Q I223V	++++	+	+
58	G7S K49R A94T S96P L111M G117S E145S N157T D173G M206Q I223V	+++++	+	+

[0095] In Table 2 above, in the stereoselectivity column, "0" indicates about 61.0-71.9% s.e. for the product (*R*)-3-hydroxythiolane, "+" indicates about 80.0-89.9% s.e. for the product, "++" indicates

about 90.0-94.9% s.e. for the product, “+++” indicates about 95.0-97.9% s.e. for the product, “++++” indicates about 98.0-98.0% s.e. for the product, and “+++++” indicates about greater than 99.0% s.e. for the product. In the activity column, “0” indicates that 1.0-4.0 g/L of KRED is needed for complete conversion of 100 g/L of ketothiolane at room temperature in more than 24 hours; “+” indicates that 0.8-1.0 g/L of KRED is needed for complete conversion of 100 g/L of ketothiolane at room temperature within 20-24 hours; and “++” indicates that 0.8-1.0 g/L of KRED is needed for complete conversion of 100 g/L of ketothiolane at room temperature within 12-20 hours. In the stability column, “-” indicates the polypeptide exhibits thermostability properties worse than wild-type; “0” indicates the polypeptide exhibits thermostability properties similar to wild-type; “+” indicates the polypeptide exhibits improved thermostability as compared to wild-type; and “++” indicates the polypeptide exhibits even more improved thermostability as compared to wild-type.

[0096] In some embodiments, an improved ketoreductase polypeptides herein comprises an amino acid sequence that is at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical as compared to a reference sequence based on SEQ ID NO: 2, 4 or 142 in which the amino acid residue corresponding to position 145 is a polar residue, particularly a serine, with the proviso that the ketoreductase polypeptides have at the residue corresponding to position 145 a polar residue, particularly a serine. In some embodiments, the ketoreductase polypeptides can have one or more residue differences at other amino acid residues as compared to the reference sequence. The differences can include substitutions, deletions, and insertions as compared to the reference sequence. The differences can be non-conservative substitutions, conservative substitutions, or a combination of non-conservative and conservative substitutions. In some embodiments, these ketoreductase polypeptides can have optionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-26, 1-30, 1-35 or about 1-40 differences at other amino acid residues. In some embodiments, the number of differences with the reference sequence can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations as compared to the reference sequence.

[0097] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence that corresponds to the sequence formulas as laid out in SEQ ID NO:143 or SEQ ID NO:144 or SEQ ID NO:145, or a domain thereof, such as residues 90-211. SEQ ID NO:145 is based on the wild-type amino acid sequence of the *L. kefir* ketoreductase (SEQ ID NO:4); SEQ ID NO:144 is based on the wild-type amino acid sequence of the *L. brevis* ketoreductase (SEQ ID NO:2); and SEQ ID NO:143 is based on the wild-type amino acid sequence of the *L. minor* ketoreductase (SEQ ID

NO:142). The ketoreductases based on the sequence formula of SEQ ID NO:143, 144 and 145 specify that residue corresponding to X145 is a polar amino acid.

[0098] In some embodiments, the ketoreductase polypeptides comprising an amino acid sequence based on sequence formulas of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, and having a polar residue, particularly serine, at the residue corresponding to X145, can further include one or more features selected from the following: residue corresponding to X3 is a polar, acidic, or aromatic residue; residue corresponding to X7 is a non-polar, polar, or constrained residue; residue corresponding to X11 is an aliphatic, non-polar, or polar residue; residue corresponding to X16 is an aliphatic or non-polar residue; residue corresponding to X19 is a non-polar or aliphatic residue; residue corresponding to X23 is a non-polar or aromatic residue; residue corresponding to X41 is an aliphatic, non-polar, or polar residue; residue corresponding to X45 is an aliphatic, non-polar, or polar residue; residue corresponding to X49 is a basic residue; residue corresponding to X57 is an aliphatic or non-polar residue; residue corresponding to X60 is an aromatic, aliphatic, non-polar, or polar residue; residue corresponding to X64 is an aliphatic or non-polar residue; residue corresponding to X72 is a basic residue; residue corresponding to X82 is a non-polar or polar residue; residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue; residue corresponding to X95 is a non-polar or aliphatic residue; residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue; residue corresponding to X97 is acidic, basic, or aliphatic residue; residue corresponding to X106 is an acidic residue; residue corresponding to X108 is a basic, constrained, or aromatic residue; residue corresponding to X111 is an aliphatic or non-polar residue; residue corresponding to X117 is a non-polar or polar residue; residue corresponding to X126 is an aliphatic or non-polar residue; residue corresponding to X127 is a polar or basic residue; residue corresponding to X147 is an aromatic, aliphatic residue, non-polar, or polar residue; residue corresponding to X152 is a polar, aliphatic or non-polar residue; residue corresponding to X157 is a polar or acidic residue; residue corresponding to X163 is an aliphatic or non-polar residue; residue corresponding to X173 is an acidic or non-polar residue; residue corresponding to X177 is a basic residue; residue corresponding to X192 is a basic or acidic residue; residue corresponding to X194 is a constrained, polar, acidic, non-polar, or aliphatic residue; residue corresponding to X198 is an acidic or non-polar residue; residue corresponding to X200 is a constrained, acidic, or basic residue; residue corresponding to X206 is a polar or non-polar residue; residue corresponding to X208 is a polar, constrained, or basic residue; residue corresponding to X210 is an aliphatic, non-polar, or polar residue; residue corresponding to X211 is a basic or acidic residue; residue corresponding to X214 is a non-polar, aliphatic or polar residue; residue corresponding to X217 is an aromatic, aliphatic, or non-polar residue; residue corresponding to X223

is an aliphatic or non-polar residue; and residue corresponding to X226 is a non-polar or aliphatic residue. In some embodiments, residue corresponding to X94 is not an aromatic residue, histidine, or glycine. In some embodiments, the residue corresponding to X96 is not an aromatic residue, glycine, isoleucine or cysteine. In some embodiments, the polypeptides comprising an amino acid sequence that corresponds to the sequence formulas as laid out in SEQ ID NO:143, 144 or 145 (or domain thereof) can have one or more of the residues not specified by an X to be conservatively mutated. In some embodiments, the conservative mutations can be 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-26, 1-30, 1-35 or about 1-40 mutations at other amino acid residues not defined by X above. In some embodiments, the number of mutations can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 amino acid residues.

[0099] In some embodiments, the polypeptides comprising an amino acid sequence based on the sequence formulas provided in SEQ ID NO:143, 144, or 145 can have one or more conservative mutations as compared to the reference sequence of SEQ ID NO: 4, 2, or 142. Exemplary conservative substitutions include amino acid replacements such as, but not limited to: replacement of residue corresponding to X19 (I) with another aliphatic residue, *e.g.*, valine; replacement of residue corresponding to X23 (I) with another non-polar residue, *e.g.*, phenylalanine; replacement of residue corresponding to X49 (K) with another basic residue, *e.g.*, arginine; replacement of residue corresponding to X57 (I) with another aliphatic residue, *e.g.*, valine; replacement of residue corresponding to X72 (K) with another basic amino acid, *e.g.*, arginine; replacement of residue corresponding to X94 (A) with another aliphatic amino acid, *e.g.*, valine; replacement of residue corresponding to X95 (A) with another aliphatic residue, *e.g.*, valine; replacement of residue corresponding to X97 (K) with another basic amino acid, *e.g.*, arginine; replacement of residue corresponding to X106 (E) with another acidic amino acid, *e.g.*, aspartic acid; replacement of residue corresponding to X111 (L) with another non-polar or aliphatic amino acid, *e.g.*, methionine; replacement of residue X147 (F) with another non-polar amino acid, *e.g.*, leucine; replacement of residue corresponding to X177 (K) with another basic amino acid, *e.g.*, arginine; replacement of residue corresponding to X192 (K) with another basic amino acid, *e.g.*, arginine; replacement of residue corresponding to X214 (M) with another non-polar amino acid, *e.g.*, valine; replacement of residue corresponding to X217 (I) with another non-polar amino acid, *e.g.*, phenylalanine; replacement of residue corresponding to X223 (I) with another non-polar or aliphatic amino acid, *e.g.*, valine; and replacement of residue corresponding to X226 (I) with another non-polar or aliphatic amino acid, *e.g.*, leucine.

[0100] In some embodiments, the ketoreductase polypeptides comprising an amino acid sequence based on sequence formulas of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211 and having a polar residue, particularly serine, at the residue corresponding to X145, can further include one or more features selected from the following: residue corresponding to X3 is asparagine, aspartic acid, or tyrosine; residue corresponding to X7 is glycine, histidine, serine or asparagine; residue corresponding to X11 is isoleucine or threonine; residue corresponding to X16 is threonine, alanine, valine, or glycine; residue corresponding to X19 is isoleucine or valine; residue corresponding to X23 is a isoleucine or phenylalanine; residue corresponding to X41 is serine, alanine, or valine; residue corresponding to X45 is glutamic acid or glycine; residue corresponding to X49 is lysine or arginine, particularly arginine; residue corresponding to X57 is isoleucine or valine; residue corresponding to X60 is phenylalanine, valine, or threonine; residue corresponding to X64 is alanine, serine, or threonine; residue corresponding to X72 is lysine or arginine, particularly arginine; residue corresponding to X82 is glycine or serine; residue corresponding to X94 is alanine, valine, threonine, serine, or arginine; residue corresponding to X95 is valine or alanine; residue corresponding to X96 is asparagine, serine, proline, alanine, or glutamic acid; residue corresponding to X97 is lysine, arginine or leucine; residue corresponding to X106 is glutamic acid or aspartic acid; residue corresponding to X108 is arginine or histidine; residue corresponding to X111 is leucine or methionine; residue corresponding to X117 is glycine or serine; residue corresponding to X126 is isoleucine or valine; residue corresponding to X127 is glutamine or arginine; residue corresponding to X147 is phenylalanine, leucine or serine; residue corresponding to X152 is threonine, serine, or methionine; residue corresponding to X157 is asparagine, glutamine, threonine, serine, or aspartic acid; residue corresponding to X163 is valine or isoleucine; residue corresponding to X173 is aspartic acid or glycine; residue corresponding to X177 is lysine or arginine; residue corresponding to X192 is lysine, arginine or glutamic acid; residue corresponding to X194 is proline, glycine, aspartic acid, arginine, or leucine; residue corresponding to X198 is aspartic acid or glycine; residue corresponding to X200 is proline, glutamic acid, or lysine; residue corresponding to X206 is methionine or glutamine; residue corresponding to X208 is glutamine, histidine or arginine; residue corresponding to X210 is threonine or alanine; residue corresponding to X211 is lysine or glutamic acid; residue corresponding to X214 is methionine, valine or threonine, or serine, particularly valine or threonine; residue corresponding to X217 is isoleucine or phenylalanine; residue corresponding to X223 is isoleucine or valine; and residue corresponding to X226 is isoleucine or valine. In some embodiments, the polypeptides comprising an amino acid sequence that corresponds to the sequence formulas as laid out in SEQ ID NO:143, 144 or 145 (or domain thereof) can have one or more of the residues not specified by an X to be conservatively mutated. In some embodiments, the conservative mutations can be 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20,

1-22, 1-24, 1-26, 1-30, 1-35 or about 1-40 mutations at other amino acid residues not defined by X above. In some embodiments, the number of mutations can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 amino acid residues.

[0101] In some embodiments, the ketoreductase polypeptides comprising an amino acid sequence based on sequence formulas of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, and having a polar residue, particularly serine, at the residue corresponding to X145, can further include one or more or at least all of the following features: residue corresponding to X7 is a non-polar, polar, or constrained residue; residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue; residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue; residue corresponding to X108 is a basic, constrained, or aromatic residue; residue corresponding to X117 is a non-polar or polar residue; residue corresponding to X157 is a polar or acidic residue; residue corresponding to X173 is an acidic or non-polar residue; residue corresponding to X206 is a polar or non-polar residue; and residue corresponding to X223 is an aliphatic or non-polar residue. In some embodiments, the amino acid residue corresponding to X145 is serine. In some embodiments, the ketoreductase polypeptides can include further include, in addition to the preceding features, one or more of features selected from the following: residue corresponding to X3 is a polar, acidic, or aromatic residue; residue corresponding to X11 is an aliphatic, non-polar, or polar residue; residue corresponding to X16 is an aliphatic or non-polar residue; residue corresponding to X19 is a non-polar or aliphatic residue; residue corresponding to X23 is a non-polar or aromatic residue; residue corresponding to X41 is an aliphatic, non-polar, or polar residue; residue corresponding to X45 is an glycine, aliphatic, non-polar, or polar residue; residue corresponding to X49 is a basic residue; residue corresponding to X57 is an aliphatic or non-polar residue; residue corresponding to X60 is an aromatic, aliphatic, non-polar, or polar residue; residue corresponding to X64 is an aliphatic or non-polar residue; residue corresponding to X72 is a basic residue; residue corresponding to X82 is a non-polar or polar residue; residue corresponding to X95 is a non-polar or aliphatic residue; residue corresponding to X97 is acidic, basic, or aliphatic residue; residue corresponding to X106 is an acidic residue; residue corresponding to X111 is an aliphatic or non-polar residue; residue corresponding to X126 is an aliphatic or non-polar residue; residue corresponding to X127 is a polar or basic residue; residue correspond to X147 is an aromatic, aliphatic residue, non-polar, or polar residue; residue corresponding to X152 is a polar, aliphatic or non-polar residue; residue corresponding to X163 is an aliphatic or non-polar residue; residue corresponding to X177 is a basic residue; residue corresponding to X192 is a basic or acidic residue; residue corresponding to X194 is a constrained, polar, non-polar, acidic, or aliphatic residue; residue corresponding to X198 is an acidic or non-polar residue; residue corresponding to X200 is a constrained, acidic, or basic

residue; residue corresponding to X208 is a polar, constrained, or basic residue; residue corresponding to X210 is an aliphatic, non-polar, or polar residue; residue corresponding to X211 is a basic or acidic residue; residue corresponding to X214 is a non-polar, aliphatic or polar residue; residue corresponding to X217 is an aromatic, aliphatic, or non-polar residue; and residue corresponding to X226 is a non-polar or aliphatic residue. In some embodiments, the polypeptides comprising an amino acid sequence that corresponds to the sequence formulas as laid out in SEQ ID NO:143, 144 or 145 (or domain thereof) can have one or more of the residues not specified by an X to be conservatively mutated. In some embodiments, the conservative mutations can be 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-26, 1-30, 1-35 or about 1-40 mutations at other amino acid residues not defined by X above. In some embodiments, the number of mutations can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 amino acid residues.

[0102] In some embodiments, the ketoreductase polypeptides comprising an amino acid sequence based on sequence formulas of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, and having a polar residue, particularly serine, at the residue corresponding to X145, can further include one or more or at least all of the features selected from the following: residue corresponding to X7 is glycine, histidine, serine or asparagine; residue corresponding to X94 is alanine, valine, threonine, serine, or arginine; residue corresponding to X96 is asparagine, serine, proline, alanine, or glutamic acid; residue corresponding to X108 is arginine or histidine; residue corresponding to X117 is glycine or serine; residue corresponding to X157 is asparagine, glutamine, threonine, serine, or aspartic acid; residue corresponding to X173 is aspartic acid or glycine; residue corresponding to X206 is methionine or glutamine; and residue corresponding to X223 is isoleucine or valine. In some embodiments, the ketoreductase polypeptides can include further include, in addition to the preceding features, one or more of features selected from the following: residue corresponding to X3 is asparagine, aspartic acid, or tyrosine; residue corresponding to X11 is isoleucine or threonine; residue corresponding to X16 is threonine, alanine, valine, or glycine; residue corresponding to X19 is isoleucine or valine; residue corresponding to X23 is an isoleucine or phenylalanine; residue corresponding to X41 is serine, alanine, or valine; residue corresponding to X45 is glutamic acid or glycine; residue corresponding to X49 is lysine or arginine, particularly arginine; residue corresponding to X57 is isoleucine or valine; residue corresponding to X60 is phenylalanine, valine, or threonine; residue corresponding to X64 is alanine, serine, or threonine; residue corresponding to X72 is lysine or arginine, particularly arginine; residue corresponding to X82 is glycine or serine; residue corresponding to X95 is valine or alanine; residue corresponding to X97 is lysine, arginine or leucine; residue corresponding to X106 is glutamic acid or aspartic acid;

residue corresponding to X111 is leucine or methionine; residue corresponding to X126 is isoleucine or valine; residue corresponding to X127 is glutamine or arginine; residue corresponding to X147 is phenylalanine, leucine or serine; residue corresponding to X152 is threonine, serine, or methionine; residue corresponding to X163 is valine or isoleucine; residue corresponding to X177 is lysine or arginine; residue corresponding to X192 is lysine, arginine or glutamic acid; residue corresponding to X194 is proline, glycine, aspartic acid, arginine, or leucine; residue corresponding to X198 is aspartic acid or glycine; residue corresponding to X200 is proline, glutamic acid, or lysine; residue corresponding to X208 is glutamine, histidine or arginine; residue corresponding to X210 is threonine or alanine; residue corresponding to X211 is lysine or glutamic acid; residue corresponding to X214 is methionine, valine or threonine, or serine, particularly valine or threonine; residue corresponding to X217 is isoleucine or phenylalanine; and residue corresponding to X226 is isoleucine or valine. In some embodiments, the polypeptides comprising an amino acid sequence that corresponds to the sequence formulas as laid out in SEQ ID NO: 143, 144 or 145 (or domain thereof) can have one or more of the residues not specified by an X to be conservatively mutated. In some embodiments, the conservative mutations can be 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-26, 1-30, 1-35 or about 1-40 mutations at other amino acid residues not defined by X above. In some embodiments, the number of mutations can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 other amino acid residues.

[0103] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine; and residue corresponding to X3 is a polar, acidic, or aromatic residue, particularly tyrosine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0104] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-

211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X7 is a non-polar, polar, or constrained residue, particularly serine, histidine, or arginine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0105] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X11 is an aliphatic, non-polar, or polar residue. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0106] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X16 is an aliphatic or non-polar residue, particularly alanine or glycine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the

preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0107] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X19 is a non-polar or aliphatic residue, particularly alanine or glycine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0108] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X23 is a non-polar or aromatic residue, particularly phenylalanine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0109] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X41 is an aliphatic, non-polar, or polar residue, particularly serine, alanine or valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0110] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X45 is an glycine, aliphatic, non-polar, or polar residue, particularly glutamic acid or glycine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0111] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X49 is a basic residue, particularly arginine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%,

86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0112] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X57 is an aliphatic or non-polar residue, particularly isoleucine or valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0113] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X60 is an aromatic, aliphatic, non-polar, or polar residue, particularly phenylalanine, valine, or threonine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of

differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0114] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X64 is an aliphatic or non-polar residue, particularly alanine, serine, or threonine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0115] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X72 is a basic residue, particularly alanine, serine, or threonine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0116] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to

X145 is a polar residue, particularly serine, and residue corresponding to X82 is a non-polar or polar residue, particularly glycine or serine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0117] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue, particularly valine, threonine, or arginine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0118] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X95 is a non-polar residue or aliphatic residue, particularly alanine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2,

1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0119] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue, particularly asparagine, serine, proline, alanine, or glutamic acid. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0120] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X97 is acidic, basic, or aliphatic residue, particularly lysine, arginine or leucine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0121] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X106 is an acidic residue, particularly aspartic acid. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0122] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly arginine or histidine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0123] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X111 is an aliphatic or non-polar residue, particularly methionine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid

sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0124] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X117 is a non-polar or polar residue, particularly serine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0125] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X126 is an aliphatic or non-polar residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18,

20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0126] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X127 is a polar or basic residue, particularly glutamine or arginine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0127] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue correspond to X147 is an aromatic, aliphatic residue, non-polar, or polar residue, particularly phenylalanine, leucine or serine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0128] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to

X145 is a polar residue, particularly serine, and residue corresponding to X152 is a polar, aliphatic or non-polar residue, non-polar, or polar residue, particularly threonine, serine, or methionine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0129] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X157 is a polar or basic residue, particularly asparagine, threonine, serine, or aspartic acid. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0130] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X163 is a an aliphatic or non-polar residue, particularly valine or isoleucine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142

having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0131] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X173 is an acidic or non-polar residue, particularly aspartic acid or glycine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0132] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X177 is a basic residue, particularly arginine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0133] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X192 is a basic or acidic residue, particularly lysine, arginine or glutamic acid. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0134] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X194 is a constrained, polar, non-polar, acidic, or aliphatic residue, particularly proline, glycine, aspartic acid, arginine, or leucine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0135] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X198 is an acidic or non-polar residue, particularly aspartic acid or glycine. In some embodiments, the ketoreductase

polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0136] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X200 is a constrained, acidic, or basic residue, particularly proline, glutamic acid, or lysine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0137] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X200 is a constrained, acidic, or basic residue, particularly proline, glutamic acid, or lysine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to

the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0138] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X206 is a polar or non-polar residue, particularly methionine or glutamine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0139] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X208 is a polar, constrained, or basic residue, particularly glutamine, histidine or arginine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0140] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to

X145 is a polar residue, particularly serine, and residue corresponding to X210 is an aliphatic, non-polar, or polar residue, particularly threonine or alanine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0141] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X211 is a basic or acidic residue, particularly lysine or glutamic acid. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0142] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X214 is a non-polar, aliphatic or polar residue, particularly valine or threonine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have

additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0143] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X217 is an aromatic, aliphatic, or non-polar residue, particularly isoleucine or phenylalanine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0144] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X223 is an aliphatic or non-polar residue, particularly isoleucine or valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0145] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X226 is a non-polar or aliphatic residue, particularly isoleucine or valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0146] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X145 is a polar residue, particularly serine, and residue corresponding to X228 is a non-polar or aliphatic residue, particularly valine or alanine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0147] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; and residue corresponding to X157 is a polar or acidic residue, particularly a threonine. In some embodiments the ketoreductases can have additionally at

residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0148] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X7 is a non-polar, polar, or constrained residue, particularly serine, histidine or asparagine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; and residue corresponding to X157 is a polar or acidic residue, particularly a threonine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0149] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue; particularly valine, threonine, or arginine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; and residue corresponding to X157 is a

polar or acidic residue, particularly a threonine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0150] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue, particularly asparagine, proline, alanine, or glutamic acid; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; and residue corresponding to X157 is a polar or acidic residue, particularly a threonine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0151] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly histidine; residue

corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; and residue corresponding to X157 is a polar or acidic residue, particularly a threonine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0152] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X173 is an acidic or non-polar residue, particularly glycine; and residue corresponding to X157 is a polar or acidic residue, particularly a threonine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0153] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to

X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar or acidic residue, particularly a threonine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0154] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X7 is a non-polar, polar, or constrained residue, particularly serine, histidine, or asparagine; residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly histidine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; and residue corresponding to X157 is a polar or acidic residue, particularly a threonine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0155] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such

as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X7 is a non-polar, polar, or constrained residue, particularly serine, histidine, or asparagine; residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue; particularly valine, threonine, or arginine; residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly histidine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; and residue corresponding to X157 is a polar or acidic residue, particularly a threonine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0156] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X7 is a non-polar, polar, or constrained residue, particularly serine, histidine, or asparagine; residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue, particularly asparagine, proline, alanine, or glutamic acid; residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly histidine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; and residue corresponding to X157 is a polar or acidic residue, particularly a threonine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30,

1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0157] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X7 is a non-polar, polar, or constrained residue, particularly serine, histidine, or asparagine; residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue; particularly valine, threonine, or arginine; residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue, particularly asparagine, proline, alanine, or glutamic acid; residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly histidine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; and residue corresponding to X157 is a polar or acidic residue, particularly a threonine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0158] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly histidine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar or acidic residue, particularly a threonine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the

ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0159] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X7 is a non-polar, polar, or constrained residue, particularly serine, histidine, or asparagine; residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly histidine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar or acidic residue, particularly a threonine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0160] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X7 is a non-polar, polar, or constrained residue, particularly serine, histidine, or asparagine; residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue; particularly valine, threonine, or arginine; residue corresponding to X108 is a basic, constrained, or aromatic

residue, particularly histidine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar or acidic residue, particularly a threonine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0161] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X7 is a non-polar, polar, or constrained residue, particularly serine, histidine, or asparagine; residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue, particularly asparagine, proline, alanine, or glutamic acid; residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly histidine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar or acidic residue, particularly a threonine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20,

22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0162] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X7 is a non-polar, polar, or constrained residue, particularly serine, histidine, or asparagine; residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue; particularly valine, threonine, or arginine; residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue, particularly asparagine, proline, alanine, or glutamic acid; residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly histidine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar or acidic residue, particularly a threonine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0163] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar or acidic residue, particularly a threonine; residue corresponding to X173 is an acidic or non-polar residue, particularly glycine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has

at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0164] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X7 is a non-polar, polar, or constrained residue, particularly serine, histidine, or asparagine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar or acidic residue, particularly a threonine; residue corresponding to X173 is an acidic or non-polar residue, particularly glycine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0165] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X7 is a non-polar, polar, or constrained residue, particularly serine, histidine, or asparagine; residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue; particularly valine, threonine, or arginine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue

corresponding to X157 is a polar or acidic residue, particularly a threonine; residue corresponding to X173 is an acidic or non-polar residue, particularly glycine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. . In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0166] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X7 is a non-polar, polar, or constrained residue, particularly serine, histidine, or asparagine; residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue, particularly asparagine, proline, alanine, or glutamic acid; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar or acidic residue, particularly a threonine; residue corresponding to X173 is an acidic or non-polar residue, particularly glycine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20,

22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0167] In some embodiments, an improved ketoreductase polypeptide comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, in which the amino acid sequence has at least the following features: residue corresponding to X7 is a non-polar, polar, or constrained residue, particularly serine, histidine, or asparagine; residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue; particularly valine, threonine, or arginine; residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue, particularly asparagine, proline, alanine, or glutamic acid; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar or acidic residue, particularly a threonine; residue corresponding to X173 is an acidic or non-polar residue, particularly glycine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments the ketoreductases can have additionally at residue corresponding to X223 an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to a reference sequence based on SEQ ID NO: 2, 4, or 142 having the preceding features. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0168] In some embodiments, an improved ketoreductase comprises an amino acid sequence that is at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a reference sequence selected from SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 86, 88, 90, 92, 94, 96, 100, 102, 104, 106, 108, 110, 112, 126, 128, 130, and 134, as listed in Table 2, wherein the improved ketoreductase polypeptide amino acid sequence includes any one set of the specified amino acid substitution combinations presented in Table 2. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 differences at other amino acid residues as compared to the reference sequence. In some embodiments, the number of differences

can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations.

[0169] In some embodiments, the improved ketoreductases comprise polypeptides with amino acid sequences corresponding to SEQ ID NOS: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 86, 88, 90, 92, 94, 96, 100, 102, 104, 106, 108, 110, 112, 126, 128, 130, and 134.

[0170] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X72 is a basic residue, particularly arginine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:104. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:104.

[0171] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly serine; residue corresponding to X108 is a constrained residue, particularly histidine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:44. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments,

the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:44.

[0172] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly serine; residue corresponding to X49 is a basic residue, particularly arginine; residue corresponding to X111 is a non-polar residue, particularly methionine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; residue corresponding to X173 is a non-polar residue, particularly glycine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:68. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:68.

[0173] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly serine; residue corresponding to X94 is a non-polar or polar residue, particularly threonine; residue corresponding to X97 is a non-polar or aliphatic residue, particularly leucine; residue corresponding to X108 is a constrained residue, particularly histidine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; residue corresponding to X223 is an aliphatic residue, particularly valine; and residue corresponding to X226 is an aliphatic residue, particularly leucine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:42. In some embodiments, the number of differences can be 1, 2,

3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:42.

[0174] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly serine; residue corresponding to X94 is a non-polar or polar residue, particularly threonine; residue corresponding to X96 is a constrained or acidic residue, particularly proline; residue corresponding to X108 is a constrained residue, particularly histidine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:24. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:24.

[0175] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly serine; residue corresponding to X94 is a non-polar or polar residue, particularly threonine; residue corresponding to X108 is a constrained residue, particularly histidine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; residue corresponding to X206 is a polar residue, particularly glutamine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:38. In some embodiments, the number

of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:38.

[0176] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly serine; residue corresponding to X94 is a non-polar or polar residue, particularly threonine; residue corresponding to X96 is a constrained or acidic residue, particularly glutamic acid; residue corresponding to X108 is a constrained residue, particularly histidine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:32. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:32.

[0177] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly arginine; residue corresponding to X94 is a non-polar or polar residue, particularly threonine; residue corresponding to X106 is an acidic residue, particularly aspartic acid; residue corresponding to X108 is a constrained residue, particularly histidine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20,

1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:8. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:8.

[0178] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X3 is an aromatic residue, particularly tyrosine; residue corresponding to X7 is a polar residue, particularly arginine; residue corresponding to X94 is a non-polar or polar residue, particularly threonine; residue corresponding to X108 is a constrained residue, particularly histidine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:134. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:134.

[0179] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly arginine; residue corresponding to X94 is a non-polar or polar residue, particularly threonine; residue corresponding to X108 is a constrained residue, particularly histidine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; residue corresponding to X208 is a constrained residue, particularly histidine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22,

1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:6. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:6.

[0180] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly arginine; residue corresponding to X94 is a non-polar or polar residue, particularly threonine; residue corresponding to X96 is a constrained residue, particularly proline; residue corresponding to X108 is a constrained residue, particularly histidine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; residue corresponding to X206 is a polar residue, particularly glutamine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:26. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:26.

[0181] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly arginine; residue corresponding to X108 is a constrained residue, particularly histidine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; residue corresponding to X206 is a polar residue, particularly glutamine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15,

1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:52. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:52.

[0182] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly arginine; residue corresponding to X96 is a constrained residue, particularly proline; residue corresponding to X108 is a constrained residue, particularly histidine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; residue corresponding to X206 is a polar residue, particularly glutamine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:50. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:50.

[0183] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly arginine; residue corresponding to X16 is a non-polar or aliphatic residue, particularly glycine; residue corresponding to X96 is a constrained residue, particularly proline; residue corresponding to X108 is a constrained residue, particularly histidine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; residue corresponding to X206 is a polar residue, particularly glutamine; and residue corresponding to X223 is an aliphatic residue,

particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:76. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:76.

[0184] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly arginine; residue corresponding to X16 is a non-polar or aliphatic residue, particularly valine; residue corresponding to X94 is a non-polar or polar residue, particularly threonine; residue corresponding to X96 is a constrained residue, particularly proline; residue corresponding to X108 is a constrained residue, particularly histidine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; residue corresponding to X206 is a polar residue, particularly glutamine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:80. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:80.

[0185] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly arginine; residue corresponding to X16 is a non-polar or aliphatic residue, particularly glycine; residue corresponding to X94 is a non-polar or polar residue, particularly threonine; residue corresponding to X96 is a constrained residue, particularly proline; residue corresponding to X108 is a

constrained residue, particularly histidine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; residue corresponding to X206 is a polar residue, particularly glutamine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:78. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:78.

[0186] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly arginine; residue corresponding to X94 is a non-polar or polar residue, particularly threonine; residue corresponding to X96 is a constrained residue, particularly proline; residue corresponding to X108 is a constrained residue, particularly histidine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; residue corresponding to X194 is a non-polar residue, particularly glycine; residue corresponding to X206 is a polar residue, particularly glutamine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:30. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:30.

[0187] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-

211, having at least the following features: residue corresponding to X7 is a polar residue, particularly arginine; residue corresponding to X94 is a non-polar or polar residue, particularly threonine; residue corresponding to X96 is a constrained residue, particularly proline; residue corresponding to X108 is a constrained residue, particularly histidine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; residue corresponding to X194 is a basic residue, particularly arginine; residue corresponding to X206 is a polar residue, particularly glutamine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:22. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:22.

[0188] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly arginine; residue corresponding to X49 is a basic residue, particularly arginine; residue corresponding to X111 is a non-polar or aliphatic residue, particularly methionine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; residue corresponding to X173 is a non-polar residue, particularly glycine; residue corresponding to X206 is a polar residue, particularly glutamine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:70. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and

wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:70.

[0189] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly arginine; residue corresponding to X49 is a basic residue, particularly arginine; residue corresponding to X96 is a constrained residue, particularly proline; residue corresponding to X111 is a non-polar or aliphatic residue, particularly methionine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; residue corresponding to X173 is a non-polar residue, particularly glycine; residue corresponding to X206 is a polar residue, particularly glutamine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at other residue positions as compared to a reference sequence of SEQ ID NO:66. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:66.

[0190] In some embodiments, an improved ketoreductase comprises an amino acid sequence based on the sequence formula of SEQ ID NO: 143, 144, or 145, or a domain thereof, such as residues 90-211, having at least the following features: residue corresponding to X7 is a polar residue, particularly arginine; residue corresponding to X49 is a basic residue, particularly arginine; residue corresponding to X94 is a polar residue, particularly threonine; residue corresponding to X96 is a constrained residue, particularly proline; residue corresponding to X111 is a non-polar or aliphatic residue, particularly methionine; residue corresponding to X117 is a polar residue, particularly serine; residue corresponding to X145 is a polar residue, particularly serine; residue corresponding to X157 is a polar residue, particularly threonine; residue corresponding to X173 is a non-polar residue, particularly glycine; residue corresponding to X206 is a polar residue, particularly glutamine; and residue corresponding to X223 is an aliphatic residue, particularly valine. In some embodiments, the ketoreductase polypeptides can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 residue differences at

other residue positions as compared to a reference sequence of SEQ ID NO:58. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 residue differences at other amino acid residues. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:58.

[0191] In some embodiments, an improved ketoreductase comprises an amino acid sequence that has a region or domain corresponding to residues 90-211 of sequence formula of SEQ ID NO: 143, 144, or 145, in which the residue corresponding to X145 in the domain is a polar residue. In some embodiments, the region or domain that corresponds to residues 90-211 of sequence formula of SEQ ID NOS: 143, 144, or 145 has at the residue corresponding to X145 a serine. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the domain of a reference sequence of based on SEQ ID NO:2, 4 or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to the amino acid sequence corresponding to residues 90-211 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding features.

[0192] In some embodiments, the ketoreductase polypeptides with a domain corresponding to residues 90-211 and having a polar residue, particularly serine, at the residue corresponding to X145, can further include one or more features selected from the following: residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue; residue corresponding to X95 is a non-polar or aliphatic residue; residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue; residue corresponding to X97 is acidic, basic, or aliphatic residue; residue corresponding to X106 is an acidic residue; residue corresponding to X108 is a basic, constrained, or aromatic residue; residue corresponding to X111 is an aliphatic or non-polar residue; residue corresponding to X117 is a non-polar or polar residue; residue corresponding to X126 is an aliphatic or non-polar residue; residue corresponding to X127 is a polar or basic residue; residue corresponding to X147 is an aromatic, aliphatic residue, non-polar, or polar residue; residue corresponding to X152 is a polar, aliphatic or non-polar residue; residue corresponding to X157 is a polar or acidic residue; residue corresponding

to X163 is an aliphatic or non-polar residue; residue corresponding to X173 is an acidic or non-polar residue; residue corresponding to X177 is a basic residue; residue corresponding to X192 is a basic or acidic residue; residue corresponding to X194 is a constrained, polar, non-polar, acidic, or aliphatic residue; residue corresponding to X198 is an acidic or non-polar residue; residue corresponding to X200 is a constrained, acidic, or basic residue; residue corresponding to X206 is a polar or non-polar residue; residue corresponding to X208 is a polar, constrained, or basic residue; residue corresponding to X210 is an aliphatic, non-polar, or polar residue; and residue corresponding to X211 is a basic or acidic residue. In some embodiments, residue corresponding to X94 is not an aromatic residue, histidine, or glycine. In some embodiments, the residue corresponding to X96 is not an aromatic residue, glycine, isoleucine or cysteine. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally from about 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the domain of a reference sequence based on SEQ ID NO:2, 4 or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations.

[0193] In some embodiments, the ketoreductase polypeptides having a domain or region corresponding to residues 90-211 based on sequence formula of SEQ ID NO:143, 144, or 145 can have one or more conservative mutations as compared to the amino acid sequence of SEQ ID NO: 4, 2, or 142. Exemplary conservative substitutions include amino acid replacements such as, but not limited to: replacement of residue corresponding to X94 (A) with another aliphatic amino acid, *e.g.*, valine; replacement of residue corresponding to X95 (V) with another non-polar or aliphatic residue, *e.g.*, alanine; replacement of residue corresponding to X97 (K) with another basic amino acid, *e.g.*, arginine; replacement of residue corresponding to X106 (E) with another acidic amino acid, *e.g.*, aspartic acid; replacement of residue corresponding to X111 (L) with another non-polar or aliphatic amino acid, *e.g.*, methionine; replacement of residue X147 (F) with another non-polar amino acid, *e.g.*, leucine; replacement of residue corresponding to X177 (K) with another basic amino acid, *e.g.*, arginine; and replacement of residue corresponding to X192 (K) with another basic amino acid, *e.g.*, arginine.

[0194] In some embodiments, the ketoreductase polypeptides with a domain corresponding to residues 90-211 and having a polar residue, particularly serine, at the residue corresponding to X145, can further include one or more features selected from the following: residue corresponding to X94 is alanine, valine, threonine, serine, or arginine; residue corresponding to X95 is valine or alanine; residue corresponding to X96 is asparagine, serine, proline, alanine, or glutamic acid; residue

corresponding to X97 is lysine, arginine or leucine; residue corresponding to X106 is glutamic acid or aspartic acid; residue corresponding to X108 is arginine or histidine; residue corresponding to X111 is leucine or methionine; residue corresponding to X117 is glycine or serine; residue corresponding to X126 is isoleucine or valine; residue corresponding to X127 is glutamine or arginine; residue corresponding to X147 is phenylalanine, leucine or serine; residue corresponding to X152 is threonine, serine, or methionine; residue corresponding to X157 is asparagine, glutamine, threonine, serine, or aspartic acid; residue corresponding to X163 is valine or isoleucine; residue corresponding to X173 is aspartic acid or glycine; residue corresponding to X177 is lysine or arginine; residue corresponding to X192 is lysine, arginine or glutamic acid; residue corresponding to X194 is proline, glycine, aspartic acid, arginine, or leucine; residue corresponding to X198 is aspartic acid or glycine; residue corresponding to X200 is proline, glutamic acid, or lysine; residue corresponding to X206 is methionine or glutamine; residue corresponding to X208 is glutamine, histidine or arginine; residue corresponding to X210 is threonine or alanine; and residue corresponding to X211 is lysine or glutamic acid. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the domain of a reference sequence based on SEQ ID NO:2, 4 or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations.

[0195] In some embodiments, the ketoreductase polypeptides with a domain corresponding to residues 90-211, and having a polar residue, particularly serine, at the residue corresponding to X145, can further include one or more or at least all of the features selected from the following: residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue; residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue; residue corresponding to X108 is a basic, constrained, or aromatic residue; residue corresponding to X117 is a non-polar or polar residue; residue corresponding to X157 is a polar or acidic residue; residue corresponding to X173 is an acidic or non-polar residue; and residue corresponding to X206 is a polar or non-polar residue. In some embodiments, the domain or region can include further include, in addition to the preceding features, one or more of the features selected from the following: residue corresponding to X97 is acidic, basic, or aliphatic residue; residue corresponding to X106 is an acidic residue; residue corresponding to X111 is an aliphatic or non-polar residue; residue corresponding to X126 is an aliphatic or non-polar residue; residue corresponding to X127 is a polar or basic residue; residue correspond to X147 is an aromatic, aliphatic residue, non-polar, or polar residue; residue corresponding to X152 is a polar, aliphatic or non-polar residue; residue corresponding to X163 is an aliphatic or non-polar residue;

residue corresponding to X177 is a basic residue; residue corresponding to X192 is a basic or acidic residue; residue corresponding to X194 is a constrained, polar, non-polar, acidic, or aliphatic residue; residue corresponding to X198 is an acidic or non-polar residue; residue corresponding to X200 is a constrained, acidic, or basic residue; residue corresponding to X208 is a polar, constrained, or basic residue; residue corresponding to X210 is an aliphatic, non-polar, or polar residue; and residue corresponding to X211 is a basic or acidic residue. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the domain of a reference sequence based on SEQ ID NO:2, 4 or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations.

[0196] In some embodiments, the ketoreductase polypeptides with a domain corresponding to residues 90-211 and having a polar residue, particularly serine, at the residue corresponding to X145, can further include one or more or at least all of the features selected from the following: residue corresponding to X94 is alanine, valine, threonine, serine, or arginine; residue corresponding to X96 is asparagine, serine, proline, alanine, or glutamic acid; residue corresponding to X108 is arginine or histidine; residue corresponding to X117 is glycine or serine; residue corresponding to X157 is asparagine, glutamine, threonine, serine, or aspartic acid; residue corresponding to X173 is aspartic acid or glycine; and residue corresponding to X206 is methionine or glutamine. In some embodiments, the domain or region can include further include, in addition to the preceding features, one or more of the features selected from the following: residue corresponding to X95 is valine or alanine; residue corresponding to X97 is lysine, arginine or leucine; residue corresponding to X106 is glutamic acid or aspartic acid; residue corresponding to X111 is leucine or methionine; residue corresponding to X126 is isoleucine or valine; residue corresponding to X127 is glutamine or arginine; residue corresponding to X147 is phenylalanine, leucine or serine; residue corresponding to X152 is threonine, serine, or methionine; residue corresponding to X163 is valine or isoleucine; residue corresponding to X173 is aspartic acid or glycine; residue corresponding to X177 is lysine or arginine; residue corresponding to X192 is lysine, arginine or glutamic acid; residue corresponding to X194 is proline, glycine, aspartic acid, arginine, or leucine; residue corresponding to X198 is aspartic acid or glycine; residue corresponding to X200 is proline, glutamic acid, or lysine; residue corresponding to X208 is glutamine, histidine or arginine; residue corresponding to X210 is threonine or alanine; and residue corresponding to X211 is lysine or glutamic acid. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7,

1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the domain of a reference sequence based on SEQ ID NO:2, 4 or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations.

[0197] In some embodiments, an improved ketoreductase comprises an amino acid sequence that has a region or domain corresponding to residues 90-211 based on the sequence formula of SEQ ID NO: 143, 144, or 145, having at least the following features: residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 a polar residue, particularly a serine; and residue corresponding to X157 is a polar or acidic residue, particularly serine or threonine. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the corresponding domain of a reference sequence based on SEQ ID NO:2, 4, or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to the amino acid sequence corresponding to residues 90-211 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding features.

[0198] In some embodiments, an improved ketoreductase comprises an amino acid sequence that has a region or domain corresponding to residues 90-211 based on the sequence formula of SEQ ID NO: 143, 144, or 145, having at least the following features: residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly histidine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 a polar residue, particularly a serine; and residue corresponding to X157 is a polar or acidic residue, particularly serine or threonine. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the domain of a reference sequence based on SEQ ID NO:2, 4, or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the

preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to the amino acid sequence corresponding to residues 90-211 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding features.

[0199] In some embodiments, an improved ketoreductase comprises an amino acid sequence that has a region or domain corresponding to residues 90-211 based on the sequence formula of SEQ ID NO: 143, 144, or 145, having at least the following features: residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue, particularly threonine or valine; residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly histidine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 a polar residue, particularly a serine; and residue corresponding to X157 is a polar or acidic residue, particularly serine or threonine. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the domain of a reference sequence based on SEQ ID NO:2, 4, or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to the amino acid sequence corresponding to residues 90-211 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding features.

[0200] In some embodiments, an improved ketoreductase comprises an amino acid sequence that has a region or domain corresponding to residues 90-211 based on the sequence formula of SEQ ID NO: 143, 144, or 145, having at least the following features: residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue, particularly asparagine, proline, alanine, or glutamic acid; residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly histidine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 a polar residue, particularly a serine; and residue corresponding to X157 is a polar or acidic residue, particularly serine or threonine. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 mutations at other amino acid residues in the domain. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally from about 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20

residue differences at other amino acid residues as compared to the domain of a reference sequence based on SEQ ID NO:2, 4, or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to the amino acid sequence corresponding to residues 90-211 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding features.

[0201] In some embodiments, an improved ketoreductase comprises an amino acid sequence that has a region or domain corresponding to residues 90-211 based on the sequence formula of SEQ ID NO: 143, 144, or 145, having at least the following features: residue corresponding to X94 is a non-polar or polar residue, particularly threonine or valine; residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue, particularly asparagine, proline, alanine, or glutamic acid; residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly histidine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 a polar residue, particularly a serine; and residue corresponding to X157 is a polar or acidic residue, particularly serine or threonine. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the domain of a reference sequence based on SEQ ID NO:2, 4, or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to the amino acid sequence corresponding to residues 90-211 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding features.

[0202] In some embodiments, an improved ketoreductase comprises an amino acid sequence that has a region or domain corresponding to residues 90-211 based on the sequence formula of SEQ ID NO: 143, 144, or 145, having at least the following features: residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly histidine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 a polar residue, particularly a serine; and residue corresponding to X157 is a polar or acidic residue, particularly serine or threonine;

and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the domain of a reference sequence based on SEQ ID NO:2, 4, or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to the amino acid sequence corresponding to residues 90-211 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding features.

[0203] In some embodiments, an improved ketoreductase comprises an amino acid sequence that has a region or domain corresponding to residues 90-211 based on the sequence formula of SEQ ID NO: 143, 144, or 145, having at least the following features: residue corresponding to X94 is a non-polar or polar residue, particularly threonine or valine; residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly histidine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 a polar residue, particularly a serine; and residue corresponding to X157 is a polar or acidic residue, particularly serine or threonine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the domain of a reference sequence based on SEQ ID NO:2, 4, or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to the amino acid sequence corresponding to residues 90-211 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding features.

[0204] In some embodiments, an improved ketoreductase comprises an amino acid sequence that has a region or domain corresponding to residues 90-211 based on the sequence formula of SEQ ID NO: 143, 144, or 145, having at least the following features: residue corresponding to X96 is a

constrained, aliphatic, non-polar, acidic, or polar residue, particularly asparagine, proline, alanine, or glutamic acid; residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly histidine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 a polar residue, particularly a serine; and residue corresponding to X157 is a polar or acidic residue, particularly serine or threonine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the domain of a reference sequence based on SEQ ID NO:2, 4, or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to the amino acid sequence corresponding to residues 90-211 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding features.

[0205] In some embodiments, an improved ketoreductase comprises an amino acid sequence that has a region or domain corresponding to residues 90-211 based on the sequence formula of SEQ ID NO: 143, 144, or 145, having at least the following features: residue corresponding to X94 is a non-polar or polar residue, particularly threonine or valine; residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue, particularly asparagine, proline, alanine, or glutamic acid; residue corresponding to X108 is a basic, constrained, or aromatic residue, particularly histidine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 a polar residue, particularly a serine; and residue corresponding to X157 is a polar or acidic residue, particularly serine or threonine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the domain of a reference sequence based on SEQ ID NO:2, 4, or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity

as compared to the amino acid sequence corresponding to residues 90-211 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding features.

[0206] In some embodiments, an improved ketoreductase comprises an amino acid sequence that has a region or domain corresponding to residues 90-211 based on the sequence formula of SEQ ID NO: 143, 144, or 145, having at least the following features: residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 a polar residue, particularly a serine; and residue corresponding to X157 is a polar or acidic residue, particularly serine or threonine; residue corresponding to X173 is an acidic or non-polar residue, particularly glycine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the domain of a reference sequence based on SEQ ID NO:2, 4, or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to the amino acid sequence corresponding to residues 90-211 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding features.

[0207] In some embodiments, an improved ketoreductase comprises an amino acid sequence that has a region or domain corresponding to residues 90-211 based on the sequence formula of SEQ ID NO: 143, 144, or 145, having at least the following features: residue corresponding to X94 is a non-polar or polar residue, particularly threonine or valine; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 a polar residue, particularly a serine; and residue corresponding to X157 is a polar or acidic residue, particularly serine or threonine; residue corresponding to X173 is an acidic or non-polar residue, particularly glycine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the domain of a reference sequence based on SEQ ID NO:2, 4, or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid

sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to the amino acid sequence corresponding to residues 90-211 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding features.

[0208] In some embodiments, an improved ketoreductase comprises an amino acid sequence that has a region or domain corresponding to residues 90-211 based on the sequence formula of SEQ ID NO: 143, 144, or 145, having at least the following features: residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue, particularly asparagine, proline, alanine, or glutamic acid; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 a polar residue, particularly a serine; and residue corresponding to X157 is a polar or acidic residue, particularly serine or threonine; residue corresponding to X173 is an acidic or non-polar residue, particularly glycine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the domain of a reference sequence based on SEQ ID NO:2, 4, or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to the amino acid sequence corresponding to residues 90-211 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding features.

[0209] In some embodiments, an improved ketoreductase comprises an amino acid sequence that has a region or domain corresponding to residues 90-211 based on the sequence formula of SEQ ID NO: 143, 144, or 145, having at least the following features: residue corresponding to X94 is a non-polar or polar residue, particularly threonine or valine; residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue, particularly asparagine, proline, alanine, or glutamic acid; residue corresponding to X117 is a non-polar or polar residue, particularly serine; residue corresponding to X145 a polar residue, particularly a serine; and residue corresponding to X157 is a polar or acidic residue, particularly serine or threonine; residue corresponding to X173 is an acidic or non-polar residue, particularly glycine; and residue corresponding to X206 is a polar or non-polar residue, particularly glutamine. In some embodiments, the region or domain corresponding to residues 90-211 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the domain of

a reference sequence based on SEQ ID NO:2, 4, or 142. In some embodiments, the number of differences can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other amino acid residues in the domain. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence with at least the preceding features, and wherein the amino acid sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to the amino acid sequence corresponding to residues 90-211 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding features.

[0210] In some embodiments, an improved ketoreductase comprises an amino acid sequence that has a region or domain corresponding to residues 1-89 of sequence formula of SEQ ID NO: 143, 144, or 145. In some embodiments, the residue corresponding to X7 in the domain is a polar or constrained residue, particularly serine, histidine, or asparagine. In some embodiments, the region or domain corresponding to residues 1-89 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues in the domain as compared to a reference sequence of SEQ ID NO:2, 4 or 142. In some embodiments, the number of difference can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences in the domain. In some embodiments, the differences comprise conservative mutations as compared to the reference sequence. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to the amino acid sequence corresponding to residues 1-89 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding feature at residue X7.

[0211] In some embodiments, the region or domain corresponding to residues 1-89 can have one or more of the features selected from the following: residue corresponding to X3 is a polar, acidic, or aromatic residue, particularly tyrosine or aspartic acid; residue corresponding to X7 is a non-polar, polar, or constrained residue, particularly serine, histidine, or asparagine; residue corresponding to X11 is an aliphatic, non-polar, or polar residue, particularly isoleucine or threonine; residue corresponding to X16 is an aliphatic or non-polar residue, particularly threonine, alanine, valine, or glycine; residue corresponding to X19 is a non-polar or aliphatic residue, particularly isoleucine or valine; residue corresponding to X23 is a nonpolar or aromatic residue, particularly phenylalanine; residue corresponding to X41 is an aliphatic, non-polar, or polar residue, particularly serine, alanine, or valine; residue corresponding to X45 is an aliphatic, non-polar, or polar residue, particularly glutamic acid or glycine; residue corresponding to X49 is a basic residue, particularly lysine or arginine; residue corresponding to X57 is an aliphatic or non-polar residue, particularly isoleucine or valine; residue corresponding to X60 is an aromatic, aliphatic, non-polar, or polar residue, particularly

phenylalanine, valine, or threonine; residue corresponding to X64 is an aliphatic or non-polar residue, particularly alanine, serine, or threonine; residue corresponding to X72 is a basic residue, particularly lysine or arginine; and residue corresponding to X82 is a non-polar or polar residue, particularly glycine or serine. In some embodiments, the region or domain corresponding to residues 1-89 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues in the domain as compared to a reference sequence of SEQ ID NO:2, 4 or 142. In some embodiments, the number of difference can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences in the domain. In some embodiments, the differences comprise conservative mutations as compared to the reference sequence. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to the amino acid sequence corresponding to residues 1-89 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding feature at residue X7.

[0212] In some embodiments, an improved ketoreductase comprises an amino acid sequence that has a region or domain corresponding to residues 212-252 of sequence formula of SEQ ID NO: 143, 144, or 145. In some embodiments, the residue corresponding to X223 in the domain is an aliphatic residue, particularly valine. In some embodiments, the region or domain corresponding to residues 212-252 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues in the domain as compared to a reference sequence of SEQ ID NO:2, 4 or 142. In some embodiments, the number of difference can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences in the domain. In some embodiments, the differences comprise conservative mutations as compared to the reference sequence. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to the amino acid sequence corresponding to residues 212-252 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding feature at residue X223.

[0213] In some embodiments, the region or domain corresponding to residues 212-252 can have one or more or at least all of the features selected from the following: residue corresponding to X214 is a non-polar, aliphatic or polar residue, particularly methionine, valine, threonine, or serine; residue corresponding to X217 is an aromatic, aliphatic, or non-polar residue, particularly isoleucine or phenylalanine; residue corresponding to X223 is an aliphatic or non-polar residue, particularly isoleucine or valine; and residue corresponding to X226 is a non-polar or aliphatic residue, particularly isoleucine or valine. In some embodiments, the region or domain corresponding to residues 212-252 can have additionally 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-

15, 1-16, 1-18, or 1-20 residue differences at other amino acid residues as compared to the domain based on reference sequence of SEQ ID NO:2, 4 or 142. In some embodiments, the number of difference can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, or about 20 residue differences at other residues in the domain. In some embodiments, the differences comprise conservative mutations. In some embodiments, the ketoreductase polypeptide comprises an amino acid sequence that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity as compared to the amino acid sequence corresponding to residues 212-252 of a reference sequence based on SEQ ID NO:2, 4 or 142 with the preceding feature at residue X223.

[0214] In some embodiments, the ketoreductase polypeptides of the present disclosure can comprise an amino acid sequence that is at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a reference sequence based on SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:142, or a region or domain thereof, such as residues 90-211, with the proviso that the residue corresponding to residue 145 is a polar residue. In some embodiments, the residue corresponding to residue 145 can be a polar residue and the polypeptide is capable of reducing the substrate to the product with at least about 70% s.e. In some embodiments, the residue corresponding to residue 145 is serine, and additionally has one or more of the following substitutions such that the polypeptide is further improved (with respect to stereoselectivity, enzymatic activity, and/or thermostability) over the wild-type kefir ketoreductase or another engineered ketoreductase: 3→Y (*i.e.*, the residue corresponding to residue 3 of SEQ ID NO:2, 4, or 142, is substituted to tyrosine); 7→S,N; 11→T; 16→A,V,G; 19→V; 23→F; 41→V; 45→G; 49→R; 57→V; 60→T; 64→T; 72→R; 82→S; 94→T,R; 95→A; 96→P,A,E; 97→R; 106→D; 108→H; 111→M; 117→S; 126→V; 127→R; 147→L,S; 152→M; 157→T,S,D; 163→I; 173→G; 177→R; 192→R,E; 194→G,D,N,L; 198→G; 200→K; 206→Q; 208→H,R; 210→A; 211→E; 214→V,T; 217→F; 223→V; 226→L; and 228→A.

[0215] In some embodiments, the residue corresponding to residue 145 is serine, and additionally has one or more of the following substitutions such that the polypeptide is further improved over the wild-type kefir ketoreductase or another engineered ketoreductase: 7→S,N; 94→T,V; 96→P; 108→H; 117→S; 157→T; 194→N; 206→Q; and 223→V.

[0216] In some embodiments, the residue corresponding to residue 145 is serine, and additionally has one or more of the following substitutions such that the polypeptide is further improved over the wild-type kefir ketoreductase or another engineered ketoreductase: 7→S,N; 94→T,V; 96→P; 108→H; 117→S; 157→T; 173→G; 194→N; 206→Q; and 223→V.

[0217] In some embodiments, each of the improved engineered ketoreductase enzymes described herein can comprise deletions of the polypeptides described herein. Thus, for each and every embodiment of the ketoreductase polypeptides of the disclosure, the deletions can be one or more amino acids, 2 or more amino acids, 3 or more amino acids, 4 or more amino acids, 5 or more amino acids, 6 or more amino acids, 8 or more amino acids, 10 or more amino acids, 15 or more amino acids, or 20 or more amino acids, up to 10% of the total number of amino acids, up to 10% of the total number of amino acids, up to 20% of the total number of amino acids, or up to 30% of the total number of amino acids of the ketoreductase polypeptides, as long as the functional activity of the ketoreductase activity is maintained. In some embodiments, the deletions can comprise, 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 1-20, 1-22, 1-24, 1-25, 1-30, 1-35 or about 1-40 amino acids. In some embodiments, the deletions can comprise deletions of 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, or 1-20 amino acid residues. In some embodiments, the number of deletions can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 18, 20, 22, 24, 26, 30, 35 or about 40 amino acids. In some embodiments, the deletions can comprise deletions of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, or 20 amino acid residues.

[0218] As will be appreciated by those of skill in the art, some of the above-defined categories of amino acid residues, unless otherwise specified, are not mutually exclusive. Thus, amino acids having side chains exhibiting two or more physico-chemical properties can be included in multiple categories. The appropriate classification of any amino acid or residue will be apparent to those of skill in the art, especially in light of the detailed disclosure provided herein.

[0219] As described herein, the ketoreductase polypeptides of the disclosure can be in the form of fusion polypeptides in which the ketoreductases polypeptides are fused to other polypeptides, such as antibody tags (*e.g.*, myc epitope) or purifications sequences (*e.g.*, His tags). Thus, the ketoreductase polypeptides can be used with or without fusions to other polypeptides.

[0220] In some embodiments, the polypeptides described herein are not restricted to the genetically encoded amino acids. In addition to the genetically encoded amino acids, the polypeptides described herein may be comprised, either in whole or in part, of naturally-occurring and/or synthetic non-encoded amino acids. Certain commonly encountered non-encoded amino acids of which the polypeptides described herein may be comprised include, but are not limited to: the D-stereoisomers of the genetically-encoded amino acids; 2,3-diaminopropionic acid (Dpr); α -aminoisobutyric acid (Aib); ϵ -aminohexanoic acid (Aha); δ -aminovaleric acid (Ava); N-methylglycine or sarcosine (MeGly or Sar); ornithine (Orn); citrulline (Cit); t-butylalanine (Bua); t-butylglycine (Bug); N-methylisoleucine (MeIle); phenylglycine (Phg); cyclohexylalanine (Cha); norleucine (Nle); naphthylalanine (Nal); 2-chlorophenylalanine (Ocf); 3-chlorophenylalanine (Mcf); 4-chlorophenylalanine (Pcf);

2-fluorophenylalanine (Off); 3-fluorophenylalanine (Mff); 4-fluorophenylalanine (Pff); 2-bromophenylalanine (Obf); 3-bromophenylalanine (Mbf); 4-bromophenylalanine (Pbf); 2-methylphenylalanine (Omf); 3-methylphenylalanine (Mmf); 4-methylphenylalanine (Pmf); 2-nitrophenylalanine (Onf); 3-nitrophenylalanine (Mnf); 4-nitrophenylalanine (Pnf); 2-cyanophenylalanine (Ocf); 3-cyanophenylalanine (Mcf); 4-cyanophenylalanine (Pcf); 2-trifluoromethylphenylalanine (Otf); 3-trifluoromethylphenylalanine (Mtf); 4-trifluoromethylphenylalanine (Ptf); 4-aminophenylalanine (Paf); 4-iodophenylalanine (Pif); 4-aminomethylphenylalanine (Pamf); 2,4-dichlorophenylalanine (Opef); 3,4-dichlorophenylalanine (Mpcf); 2,4-difluorophenylalanine (Opff); 3,4-difluorophenylalanine (Mpff); pyrid-2-ylalanine (2pAla); pyrid-3-ylalanine (3pAla); pyrid-4-ylalanine (4pAla); naphth-1-ylalanine (1nAla); naphth-2-ylalanine (2nAla); thiazolylalanine (taAla); benzothienylalanine (bAla); thienylalanine (tAla); furylalanine (fAla); homophenylalanine (hPhe); homotyrosine (hTyr); homotryptophan (hTrp); pentafluorophenylalanine (5ff); styrylalanine (sAla); authrylalanine (aAla); 3,3-diphenylalanine (Dfa); 3-amino-5-phenylpentanoic acid (Afp); penicillamine (Pen); 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic); β -2-thienylalanine (Thi); methionine sulfoxide (Mso); N(w)-nitroarginine (nArg); homolysine (hLys); phosphonomethylphenylalanine (pmPhe); phosphoserine (pSer); phosphothreonine (pThr); homoaspartic acid (hAsp); homoglutamic acid (hGlu); 1-aminocyclopent-(2 or 3)-ene-4 carboxylic acid; pipercolic acid (PA), azetidine-3-carboxylic acid (ACA); 1-aminocyclopentane-3-carboxylic acid; allylglycine (aOly); propargylglycine (pgGly); homoalanine (hAla); norvaline (nVal); homoleucine (hLeu), homovaline (hVal); homoisoleucine (hIle); homoarginine (hArg); N-acetyl lysine (AcLys); 2,4-diaminobutyric acid (Dbu); 2,3-diaminobutyric acid (Dab); N-methylvaline (MeVal); homocysteine (hCys); homoserine (hSer); hydroxyproline (Hyp) and homoproline (hPro). Additional non-encoded amino acids of which the polypeptides described herein may be comprised will be apparent to those of skill in the art (see, *e.g.*, the various amino acids provided in Fasman, 1989, CRC Practical Handbook of Biochemistry and Molecular Biology, CRC Press, Boca Raton, FL, at pp. 3-70 and the references cited therein, all of which are incorporated by reference). These amino acids may be in either the L- or D-configuration.

[0221] Those of skill in the art will recognize that amino acids or residues bearing side chain protecting groups may also comprise the polypeptides described herein. Non-limiting examples of such protected amino acids, which in this case belong to the aromatic category, include (protecting groups listed in parentheses), but are not limited to: Arg(tos), Cys(methylbenzyl), Cys(nitropyridinesulfenyl), Glu(δ -benzylester), Gln(xanthyl), Asn(N- δ -xanthyl), His(bom), His(benzyl), His(tos), Lys(fmoc), Lys(tos), Ser(O-benzyl), Thr (O-benzyl) and Tyr(O-benzyl).

[0222] Non-encoding amino acids that are conformationally constrained of which the polypeptides described herein may be composed include, but are not limited to, N-methyl amino acids (L-configuration); 1-aminocyclopent-(2 or 3)-ene-4-carboxylic acid; pipecolic acid; azetidine-3-carboxylic acid; homoproline (hPro); and 1-aminocyclopentane-3-carboxylic acid.

7.3 Polynucleotides Encoding Engineered Ketoreductases

[0223] In another aspect, the present disclosure provides polynucleotides encoding the engineered ketoreductase enzymes. The polynucleotides may be operatively linked to one or more heterologous regulatory sequences that control gene expression to create a recombinant polynucleotide capable of expressing the polypeptide. Expression constructs containing a heterologous polynucleotide encoding the engineered ketoreductase can be introduced into appropriate host cells to express the corresponding ketoreductase polypeptide.

[0224] Because of the knowledge of the codons corresponding to the various amino acids, availability of a protein sequence provides a description of all the polynucleotides capable of encoding the subject. The degeneracy of the genetic code, where the same amino acids are encoded by alternative or synonymous codons allows an extremely large number of nucleic acids to be made, all of which encode the improved ketoreductase enzymes disclosed herein. Thus, having identified a particular amino acid sequence, those skilled in the art could make any number of different nucleic acids by simply modifying the sequence of one or more codons in a way which does not change the amino acid sequence of the protein. In this regard, the present disclosure specifically contemplates each and every possible variation of polynucleotides that could be made by selecting combinations based on the possible codon choices, and all such variations are to be considered specifically disclosed for any polypeptide disclosed herein, including the amino acid sequences presented in Table 2.

[0225] In various embodiments, the codons are preferably selected to fit the host cell in which the protein is being produced. For example, preferred codons used in bacteria are used to express the gene in bacteria; preferred codons used in yeast are used for expression in yeast; and preferred codons used in mammals are used for expression in mammalian cells. By way of example, the polynucleotide of SEQ ID NO: 3 has been codon optimized for expression in *E. coli*, but otherwise encodes the naturally occurring ketoreductase of *L. kefir*.

[0226] In some embodiments, the polynucleotide comprises a nucleotide sequence encoding a ketoreductase polypeptide with an amino acid sequence that has at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more sequence identity to any of the reference engineered ketoreductase polypeptides described herein, where the encoded

ketoreductase polypeptide comprises an amino acid sequence that has at the residue corresponding to residue position 145 of SEQ ID NO:2, 4 or 142 a polar residue, particularly a serine. In some embodiments, the polynucleotides encode an engineered ketoreductase polypeptide comprising an amino acid sequence selected from SEQ ID NOS: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 64, 66, 68, 70, 74, 76, 78, 80, 86, 88, 90, 92, 104, 106, 110, 112, 124, 126, 130, or 134.

[0227] In some embodiments, the polynucleotides encoding the engineered ketoreductases are selected from SEQ ID NO: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, and 133.

[0228] In some embodiments, the polynucleotides are capable of hybridizing under highly stringent conditions to one of the polynucleotides comprising a sequence corresponding to SEQ ID NO:5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, and 133, where the polynucleotide that hybridizes under highly stringent conditions encode a functional ketoreductase capable of converting the substrate of structural formula (I) to the product of structural formula (II).

[0229] In some embodiments, the polynucleotides comprise a nucleic acid sequence that encodes the polypeptides described herein but have about 80% or more sequence identity, about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more sequence identity at the nucleotide level to a reference polynucleotide encoding an engineered ketoreductase, where the polynucleotide encodes a functional ketoreductase capable of converting the substrate of structural formula (I) to the product of structural formula (II). In some embodiments, the reference polynucleotide is selected from the sequences corresponding to SEQ ID NO: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, and 133.

[0230] An isolated polynucleotide encoding an improved ketoreductase polypeptide may be manipulated in a variety of ways to provide for expression of the polypeptide. Manipulation of the isolated polynucleotide prior to its insertion into a vector may be desirable or necessary depending on the expression vector. The techniques for modifying polynucleotides and nucleic acid sequences utilizing recombinant DNA methods are well known in the art. Guidance is provided in Sambrook et al., 2001, *Molecular Cloning: A Laboratory Manual*, 3rd Ed., Cold Spring Harbor Laboratory Press;

and *Current Protocols in Molecular Biology*, Ausubel, F. ed., Greene Pub. Associates, 1998, updates to 2006.

[0231] For bacterial host cells, suitable promoters for directing transcription of the nucleic acid constructs of the present disclosure, include the promoters obtained from the *E. coli* lac operon, *Streptomyces coelicolor* agarase gene (*dagA*), *Bacillus subtilis* levansucrase gene (*sacB*), *Bacillus licheniformis* alpha-amylase gene (*amyL*), *Bacillus stearothermophilus* maltogenic amylase gene (*amyM*), *Bacillus amyloliquefaciens* alpha-amylase gene (*amyQ*), *Bacillus licheniformis* penicillinase gene (*penP*), *Bacillus subtilis* *xylA* and *xylB* genes, and prokaryotic β -lactamase gene (Villa-Kamaroff et al., 1978, *Proc. Natl Acad. Sci. USA* 75: 3727-3731), as well as the *tac* promoter (DeBoer et al., 1983, *Proc. Natl Acad. Sci. USA* 80: 21-25). Further promoters are described in "Useful proteins from recombinant bacteria" in *Scientific American*, 1980, 242:74-94; and in Sambrook et al., *supra*.

[0232] For filamentous fungal host cells, suitable promoters for directing the transcription of the nucleic acid constructs of the present disclosure include promoters obtained from the genes for *Aspergillus oryzae* TAKA amylase, *Rhizomucor miehei* aspartic proteinase, *Aspergillus niger* neutral alpha-amylase, *Aspergillus niger* acid stable alpha-amylase, *Aspergillus niger* or *Aspergillus awamori* glucoamylase (*glaA*), *Rhizomucor miehei* lipase, *Aspergillus oryzae* alkaline protease, *Aspergillus oryzae* triose phosphate isomerase, *Aspergillus nidulans* acetamidase, and *Fusarium oxysporum* trypsin-like protease (WO 96/00787), as well as the NA2-*tpi* promoter (a hybrid of the promoters from the genes for *Aspergillus niger* neutral alpha-amylase and *Aspergillus oryzae* triose phosphate isomerase), and mutant, truncated, and hybrid promoters thereof.

[0233] In a yeast host, useful promoters can be from the genes for *Saccharomyces cerevisiae* enolase (ENO-1), *Saccharomyces cerevisiae* galactokinase (GAL1), *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (ADH2/GAP), and *Saccharomyces cerevisiae* 3-phosphoglycerate kinase. Other useful promoters for yeast host cells are described by Romanos et al., 1992, *Yeast* 8:423-488.

[0234] The control sequence may also be a suitable transcription terminator sequence, a sequence recognized by a host cell to terminate transcription. The terminator sequence is operably linked to the 3' terminus of the nucleic acid sequence encoding the polypeptide. Any terminator which is functional in the host cell of choice may be used in the present invention.

[0235] For example, exemplary transcription terminators for filamentous fungal host cells can be obtained from the genes for *Aspergillus oryzae* TAKA amylase, *Aspergillus niger* glucoamylase,

Aspergillus nidulans anthranilate synthase, *Aspergillus niger* alpha-glucosidase, and *Fusarium oxysporum* trypsin-like protease.

[0236] Exemplary terminators for yeast host cells can be obtained from the genes for *Saccharomyces cerevisiae* enolase, *Saccharomyces cerevisiae* cytochrome C (CYC1), and *Saccharomyces cerevisiae* glyceraldehyde-3-phosphate dehydrogenase. Other useful terminators for yeast host cells are described by Romanos et al., 1992, *supra*.

[0237] The control sequence may also be a suitable leader sequence, a nontranslated region of an mRNA that is important for translation by the host cell. The leader sequence is operably linked to the 5' terminus of the nucleic acid sequence encoding the polypeptide. Any leader sequence that is functional in the host cell of choice may be used. Exemplary leaders for filamentous fungal host cells are obtained from the genes for *Aspergillus oryzae* TAKA amylase and *Aspergillus nidulans* triose phosphate isomerase. Suitable leaders for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* enolase (ENO-1), *Saccharomyces cerevisiae* 3-phosphoglycerate kinase, *Saccharomyces cerevisiae* alpha-factor, and *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (ADH2/GAP).

[0238] The control sequence may also be a polyadenylation sequence, a sequence operably linked to the 3' terminus of the nucleic acid sequence and which, when transcribed, is recognized by the host cell as a signal to add polyadenosine residues to transcribed mRNA. Any polyadenylation sequence which is functional in the host cell of choice may be used in the present invention. Exemplary polyadenylation sequences for filamentous fungal host cells can be from the genes for *Aspergillus oryzae* TAKA amylase, *Aspergillus niger* glucoamylase, *Aspergillus nidulans* anthranilate synthase, *Fusarium oxysporum* trypsin-like protease, and *Aspergillus niger* alpha-glucosidase. Useful polyadenylation sequences for yeast host cells are described by Guo and Sherman, 1995, *Mol Cell Bio.* 15:5983-5990.

[0239] The control sequence may also be a signal peptide coding region that codes for an amino acid sequence linked to the amino terminus of a polypeptide and directs the encoded polypeptide into the cell's secretory pathway. The 5' end of the coding sequence of the nucleic acid sequence may inherently contain a signal peptide coding region naturally linked in translation reading frame with the segment of the coding region that encodes the secreted polypeptide. Alternatively, the 5' end of the coding sequence may contain a signal peptide coding region that is foreign to the coding sequence. The foreign signal peptide coding region may be required where the coding sequence does not naturally contain a signal peptide coding region.

[0240] Alternatively, the foreign signal peptide coding region may simply replace the natural signal peptide coding region in order to enhance secretion of the polypeptide. However, any signal peptide coding region which directs the expressed polypeptide into the secretory pathway of a host cell of choice may be used in the present invention.

[0241] Effective signal peptide coding regions for bacterial host cells are the signal peptide coding regions obtained from the genes for *Bacillus* NCIB 11837 maltogenic amylase, *Bacillus stearothermophilus* alpha-amylase, *Bacillus licheniformis* subtilisin, *Bacillus licheniformis* β -lactamase, *Bacillus stearothermophilus* neutral proteases (nprT, nprS, nprM), and *Bacillus subtilis* prsA. Further signal peptides are described by Simonen and Palva, 1993, *Microbiol Rev* 57: 109-137.

[0242] Effective signal peptide coding regions for filamentous fungal host cells can be the signal peptide coding regions obtained from the genes for *Aspergillus oryzae* TAKA amylase, *Aspergillus niger* neutral amylase, *Aspergillus niger* glucoamylase, *Rhizomucor miehei* aspartic proteinase, *Humicola insolens* cellulase, and *Humicola lanuginosa* lipase.

[0243] Useful signal peptides for yeast host cells can be from the genes for *Saccharomyces cerevisiae* alpha-factor and *Saccharomyces cerevisiae* invertase. Other useful signal peptide coding regions are described by Romanos et al., 1992, *supra*.

[0244] The control sequence may also be a propeptide coding region that codes for an amino acid sequence positioned at the amino terminus of a polypeptide. The resultant polypeptide is known as a proenzyme or propolypeptide (or a zymogen in some cases). A propolypeptide is generally inactive and can be converted to a mature active polypeptide by catalytic or autocatalytic cleavage of the propeptide from the propolypeptide. The propeptide coding region may be obtained from the genes for *Bacillus subtilis* alkaline protease (aprE), *Bacillus subtilis* neutral protease (nprT), *Saccharomyces cerevisiae* alpha-factor, *Rhizomucor miehei* aspartic proteinase, and *Myceliophthora thermophila* lactase (WO 95/33836).

[0245] Where both signal peptide and propeptide regions are present at the amino terminus of a polypeptide, the propeptide region is positioned next to the amino terminus of a polypeptide and the signal peptide region is positioned next to the amino terminus of the propeptide region.

[0246] It may also be desirable to add regulatory sequences, which allow the regulation of the expression of the polypeptide relative to the growth of the host cell. Examples of regulatory systems are those which cause the expression of the gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. In prokaryotic host cells, suitable regulatory sequences include the lac, tac, and trp operator systems. In yeast host cells, suitable regulatory systems include, as examples, the ADH2 system or GAL1 system. In filamentous

fungi, suitable regulatory sequences include the TAKA alpha-amylase promoter, *Aspergillus niger* glucoamylase promoter, and *Aspergillus oryzae* glucoamylase promoter.

[0247] Other examples of regulatory sequences are those which allow for gene amplification. In eukaryotic systems, these include the dihydrofolate reductase gene, which is amplified in the presence of methotrexate, and the metallothionein genes, which are amplified with heavy metals. In these cases, the nucleic acid sequence encoding the KRED polypeptide of the present invention would be operably linked with the regulatory sequence.

[0248] Thus, in some embodiments, the present disclosure is also directed to a recombinant expression vector comprising a polynucleotide encoding an engineered ketoreductase polypeptide or a variant thereof, and one or more expression regulating regions such as a promoter and a terminator, a replication origin, etc., depending on the type of hosts into which they are to be introduced. The various nucleic acid and control sequences described above may be joined together to produce a recombinant expression vector which may include one or more convenient restriction sites to allow for insertion or substitution of the nucleic acid sequence encoding the polypeptide at such sites. Alternatively, the nucleic acid sequence of the present disclosure may be expressed by inserting the nucleic acid sequence or a nucleic acid construct comprising the sequence into an appropriate vector for expression. In creating the expression vector, the coding sequence is located in the vector so that the coding sequence is operably linked with the appropriate control sequences for expression.

[0249] The recombinant expression vector may be any vector (*e.g.*, a plasmid or virus), which can be conveniently subjected to recombinant DNA procedures and can bring about the expression of the polynucleotide sequence. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vectors may be linear or closed circular plasmids.

[0250] In some embodiments, the expression vector may be an autonomously replicating vector, *i.e.*, a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.*, a plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. Furthermore, a single vector or plasmid or two or more vectors or plasmids which together contain the total DNA to be introduced into the genome of the host cell, or a transposon may be used.

[0251] The expression vectors of the present disclosure can contain one or more selectable markers, which permit easy selection of transformed cells. A selectable marker is a gene the product of which

provides for biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs, and the like. Examples of bacterial selectable markers are the *dal* genes from *Bacillus subtilis* or *Bacillus licheniformis*, or markers, which confer antibiotic resistance such as ampicillin, kanamycin, chloramphenicol (Example 1) or tetracycline resistance. Suitable markers for yeast host cells are ADE2, HIS3, LEU2, LYS2, MET3, TRP1, and URA3.

[0252] Selectable markers for use in a filamentous fungal host cell include, but are not limited to, *amdS* (acetamidase), *argB* (ornithine carbamoyltransferase), *bar* (phosphinothricin acetyltransferase), *hph* (hygromycin phosphotransferase), *niaD* (nitrate reductase), *pyrG* (orotidine-5'-phosphate decarboxylase), *sC* (sulfate adenylyltransferase), and *trpC* (anthranilate synthase), as well as equivalents thereof. Embodiments for use in an *Aspergillus* cell include the *amdS* and *pyrG* genes of *Aspergillus nidulans* or *Aspergillus oryzae* and the *bar* gene of *Streptomyces hygroscopicus*.

[0253] The expression vectors of the present invention preferably contain an element(s) that permits integration of the vector into the host cell's genome or autonomous replication of the vector in the cell independent of the genome. For integration into the host cell genome, the vector may rely on the nucleic acid sequence encoding the polypeptide or any other element of the vector for integration of the vector into the genome by homologous or nonhomologous recombination.

[0254] Alternatively, the expression vector may contain additional nucleic acid sequences for directing integration by homologous recombination into the genome of the host cell. The additional nucleic acid sequences enable the vector to be integrated into the host cell genome at a precise location(s) in the chromosome(s). To increase the likelihood of integration at a precise location, the integrational elements should preferably contain a sufficient number of nucleic acids, such as 100 to 10,000 base pairs, preferably 400 to 10,000 base pairs, and most preferably 800 to 10,000 base pairs, which are highly homologous with the corresponding target sequence to enhance the probability of homologous recombination. The integrational elements may be any sequence that is homologous with the target sequence in the genome of the host cell. Furthermore, the integrational elements may be non-encoding or encoding nucleic acid sequences. On the other hand, the vector may be integrated into the genome of the host cell by non-homologous recombination.

[0255] For autonomous replication, the vector may further comprise an origin of replication enabling the vector to replicate autonomously in the host cell in question. Examples of bacterial origins of replication are P15A ori or the origins of replication of plasmids pBR322, pUC19, pACYCI77 (which plasmid has the P15A ori), or pACYC184 permitting replication in *E. coli*, and pUBI10, pE194, pTA1060, or pAM β 1 permitting replication in *Bacillus*. Examples of origins of replication for use in a yeast host cell are the 2 micron origin of replication, ARS1, ARS4, the combination of ARS1 and

CEN3, and the combination of ARS4 and CEN6. The origin of replication may be one having a mutation which makes its functioning temperature-sensitive in the host cell (see, *e.g.*, Ehrlich, 1978, *Proc Natl Acad Sci. USA* 75:1433).

[0256] More than one copy of a nucleic acid sequence of the present invention may be inserted into the host cell to increase production of the gene product. An increase in the copy number of the nucleic acid sequence can be obtained by integrating at least one additional copy of the sequence into the host cell genome or by including an amplifiable selectable marker gene with the nucleic acid sequence where cells containing amplified copies of the selectable marker gene, and thereby additional copies of the nucleic acid sequence, can be selected for by cultivating the cells in the presence of the appropriate selectable agent.

[0257] Many of the expression vectors for use in the present invention are commercially available. Suitable commercial expression vectors include p3xFLAGTM™ expression vectors from Sigma-Aldrich Chemicals, St. Louis MO., which includes a CMV promoter and hGH polyadenylation site for expression in mammalian host cells and a pBR322 origin of replication and ampicillin resistance markers for amplification in *E. coli*. Other suitable expression vectors are pBluescriptII SK(-) and pBK-CMV, which are commercially available from Stratagene, LaJolla CA, and plasmids which are derived from pBR322 (Gibco BRL), pUC (Gibco BRL), pREP4, pCEP4 (Invitrogen) or pPoly (Lathe et al., 1987, *Gene* 57:193-201).

7.4 Host Cells for Expression of Ketoreductase Polypeptides

[0258] In another aspect, the present disclosure provides a host cell comprising a polynucleotide encoding an improved ketoreductase polypeptide of the present disclosure, the polynucleotide being operatively linked to one or more control sequences for expression of the ketoreductase enzyme in the host cell. Host cells for use in expressing the KRED polypeptides encoded by the expression vectors of the present invention are well known in the art and include but are not limited to, bacterial cells, such as *E. coli*, *Lactobacillus kefir*, *Lactobacillus brevis*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells (*e.g.*, *Saccharomyces cerevisiae* or *Pichia pastoris* (ATCC Accession No. 201178)); insect cells such as *Drosophila* S2 and *Spodoptera Sf9* cells; animal cells such as CHO, COS, BHK, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and growth conditions for the above-described host cells are well known in the art.

[0259] Polynucleotides for expression of the ketoreductase may be introduced into cells by various methods known in the art. Techniques include, among others, electroporation, biolistic particle bombardment, liposome mediated transfection, calcium chloride transfection, and protoplast fusion. Various methods for introducing polynucleotides into cells will be apparent to the skilled artisan.

[0260] An exemplary host cell is *Escherichia coli* W3110. The expression vector was created by operatively linking a polynucleotide encoding an improved ketoreductase into the plasmid pCK110900 operatively linked to the lac promoter under control of the lacI repressor. The expression vector also contains the P15a origin of replication and the chloramphenicol resistance gene. Cells containing the subject polynucleotide in *Escherichia coli* W3110 were isolated by subjecting the cells to chloramphenicol selection.

7.5 Methods of Generating Engineered Ketoreductase Polypeptides.

[0261] In some embodiments, to make the improved KRED polynucleotides and polypeptides of the present disclosure, the naturally-occurring ketoreductase enzyme that catalyzes the reduction reaction is obtained (or derived) from *Lactobacillus kefir* or *Lactobacillus brevis*. In some embodiments, the parent polynucleotide sequence is codon optimized to enhance expression of the ketoreductase in a specified host cell. As an illustration, the parental polynucleotide sequence encoding the wild-type KRED polypeptide of *Lactobacillus kefir* was constructed from oligonucleotides prepared based upon the known polypeptide sequence of *Lactobacillus kefir* KRED sequence available in Genbank database (Genbank accession no. AAP94029 GI:33112056). The parental polynucleotide sequence was codon optimized for expression in *E. coli* and the codon-optimized polynucleotide cloned into an expression vector, placing the expression of the ketoreductase gene under the control of the lac promoter and lacI repressor gene. Clones expressing the active ketoreductase in *E. coli* were identified and the genes sequenced to confirm their identity. The sequence designated (SEQ ID NO: 1) was the parent sequence utilized as the starting point for most experiments and library construction of engineered ketoreductases evolved from the *Lactobacillus kefir* ketoreductase.

[0262] The engineered ketoreductases can be obtained by subjecting the polynucleotide encoding the naturally occurring ketoreductase to mutagenesis and/or directed evolution methods, as discussed above. An exemplary directed evolution technique is mutagenesis and/or DNA shuffling as described in Stemmer, 1994, *Proc Natl Acad Sci USA* 91:10747-10751; WO 95/22625; WO 97/0078; WO 97/35966; WO 98/27230; WO 00/42651; WO 01/75767 and U.S. Pat. 6,537,746. Other directed evolution procedures that can be used include, among others, staggered extension process (StEP), in vitro recombination (Zhao et al., 1998, *Nat. Biotechnol.* 16:258-261), mutagenic PCR (Caldwell et al., 1994, *PCR Methods Appl.* 3:S136-S140), and cassette mutagenesis (Black et al., 1996, *Proc Natl Acad Sci USA* 93:3525-3529).

[0263] The clones obtained following mutagenesis treatment are screened for engineered ketoreductases having a desired improved enzyme property. Measuring enzyme activity from the expression libraries can be performed using the standard biochemistry technique of monitoring the

rate of decrease (via a decrease in absorbance or fluorescence) of NADH or NADPH concentration, as it is converted into NAD^+ or NADP^+ . (For example, see Example 7.) In this reaction, the NADH or NADPH is consumed (oxidized) by the ketoreductase as the ketoreductase reduces a ketone substrate to the corresponding hydroxyl group. The rate of decrease of NADH or NADPH concentration, as measured by the decrease in absorbance or fluorescence, per unit time indicates the relative (enzymatic) activity of the KRED polypeptide in a fixed amount of the lysate (or a lyophilized powder made therefrom). Where the improved enzyme property desired is thermal stability, enzyme activity may be measured after subjecting the enzyme preparations to a defined temperature and measuring the amount of enzyme activity remaining after heat treatments. Clones containing a polynucleotide encoding a ketoreductase are then isolated, sequenced to identify the nucleotide sequence changes (if any), and used to express the enzyme in a host cell.

[0264] Where the sequence of the engineered polypeptide is known, the polynucleotides encoding the enzyme can be prepared by standard solid-phase methods, according to known synthetic methods. In some embodiments, fragments of up to about 100 bases can be individually synthesized, then joined (*e.g.*, by enzymatic or chemical ligation methods, or polymerase mediated methods) to form any desired continuous sequence. For example, polynucleotides and oligonucleotides of the invention can be prepared by chemical synthesis using, *e.g.*, the classical phosphoramidite method described by Beaucage et al., 1981, *Tet Lett* 22:1859-69, or the method described by Matthes et al., 1984, *EMBO J.* 3:801-05, *e.g.*, as it is typically practiced in automated synthetic methods. According to the phosphoramidite method, oligonucleotides are synthesized, *e.g.*, in an automatic DNA synthesizer, purified, annealed, ligated and cloned in appropriate vectors. In addition, essentially any nucleic acid can be obtained from any of a variety of commercial sources, such as The Midland Certified Reagent Company, Midland, TX, The Great American Gene Company, Ramona, CA, ExpressGen Inc. Chicago, IL, Operon Technologies Inc., Alameda, CA, and many others.

[0265] Engineered ketoreductase enzymes expressed in a host cell can be recovered from the cells and or the culture medium using any one or more of the well known techniques for protein purification, including, among others, lysozyme treatment, sonication, filtration, salting-out, ultra-centrifugation, and chromatography. Suitable solutions for lysing and the high efficiency extraction of proteins from bacteria, such as *E. coli*, are commercially available under the trade name CellLytic BTM from Sigma-Aldrich of St. Louis MO.

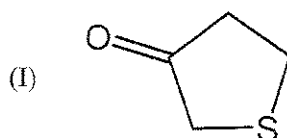
[0266] Chromatographic techniques for isolation of the ketoreductase polypeptide include, among others, reverse phase chromatography, high performance liquid chromatography, ion exchange chromatography, gel electrophoresis, and affinity chromatography. Conditions for purifying a

particular enzyme will depend, in part, on factors such as net charge, hydrophobicity, hydrophilicity, molecular weight, molecular shape, etc., and will be apparent to those having skill in the art.

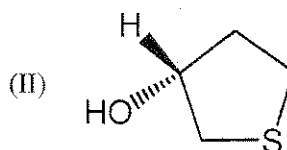
[0267] In some embodiments, affinity techniques may be used to isolate the improved ketoreductase enzymes. For affinity chromatography purification, any antibody which specifically binds the ketoreductase polypeptide may be used. For the production of antibodies, various host animals, including but not limited to rabbits, mice, rats, etc., may be immunized by injection with a polypeptide. The polypeptide may be attached to a suitable carrier, such as BSA, by means of a side chain functional group or linkers attached to a side chain functional group. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacilli Calmette Guerin) and Corynebacterium parvum.

7.6 Methods of Using the Engineered Ketoreductase Enzymes and Compounds Prepared Therewith

[0268] The ketoreductase enzymes described herein are capable of catalyzing the reduction reaction of the keto group in the compound of structural formula (I), 3-ketothiolane:



to the corresponding chiral alcohol product of structural formula (II), (R)-3-hydroxythiolane:



[0269] In some embodiments, the invention provides a method for reducing a substrate of structural compound of formula (I) to the product of the structural formula (II), where the method comprises contacting or incubating the substrate with a ketoreductase polypeptide disclosed herein under reaction conditions suitable for reducing the substrate to the alcohol product.

[0270] For example, in some embodiments of the method, the ketoreductase polypeptides comprise an amino acid sequence having, as compared to the wild-type *L. kefir* or *L. brevis* or *L. minor* KRED

sequences of SEQ ID NO: 4, 2 and 142, respectively, a polar residue, particularly a serine, at the residue corresponding to X145. As noted herein, the ketoreductase polypeptide can have in addition, one or more mutations at other amino acid residues as compared to the references sequences of SEQ ID NO: 2, 4, or 106. Such differences are described in the sequence formulas of SEQ ID NO: 143, 144, and 145 and the descriptions herein.

[0271] In some embodiments of this method, the ketoreductase polypeptide comprises, by way of example and not limitation, an amino acid sequence that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a reference sequence based on SEQ ID NO: 2, 4, or 142 having a polar residue, particularly serine, at the residue corresponding to X145, where the ketoreductase polypeptide also has a polar residue, particularly serine, at the residue corresponding to X145.

[0272] In some embodiments of the method, the product is reduced in greater than about 65% stereomeric excess, where the ketoreductase polypeptide comprises a sequence corresponding to SEQ ID NOS: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 64, 66, 68, 70, 74, 76, 78, 80, 86, 88, 90, 92, 104, 106, 110, 112, 124, 126, 130, or 134.

[0273] In some embodiments of the method, the substrate is reduced to the product in greater than about 90% stereomeric excess, wherein the ketoreductase polypeptide comprises an amino acid sequences corresponding to SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 86, 88, 90, 92, 94, 96, 100, 102, 104, 106, 108, 110, 112, 126, 128, 130, or 134.

[0274] In some embodiments of the method, the substrate is reduced to the product in greater than about 98% s.e., wherein the ketoreductase polypeptide comprises an amino acid sequences corresponding to corresponding to SEQ ID NO: 6, 8, 10, 18, 20, 22, 24, 26, 28, 30, 34, 36, 38, 40, 42, 50, 52, 54, 58, 62, 66, 70, 72, 76, 78, 80, and 134.

[0275] In some embodiments of the method for reducing the substrate to the product, the substrate is reduced to the product at an improved rate as compared to the wild-type enzyme (SEQ ID NO: 4), wherein the ketoreductase polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 64, 66, 68, 70, 74, 76, 78, 80, 86, 88, 90, 92, 104, 106, 110, 112, 124, 126, 130, or 134.

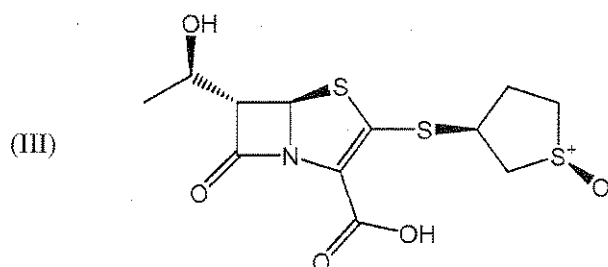
[0276] In some embodiments, the reaction condition is pH 7.5 or less. In some embodiments, the reaction condition is at a pH of from about 5.0 to about 7.5. In some embodiments, the reaction condition is at a pH of from about pH 6.0 to 7.5. In some embodiments, the reaction condition temperature is about 25°C or less. In some embodiments, the reaction condition is at a temperature of

5°C to about 25°C or 5°C to 15°C. In some embodiments, the reaction condition also includes a cofactor regenerating system, as further described below.

[0277] In some embodiments, a method for reducing the substrate to the product can comprise contacting the substrate with a ketoreductase of the disclosure in a reaction condition comprising at least 100 g/L of substrate and at least 0.8-1.0 g/L of the ketoreductase polypeptide, wherein at least 90% of the substrate is converted to product in less than 24 hrs (*e.g.*, 20-24 hrs). In some embodiments, greater than 95%, 96%, 97%, 98%, or 99% or more of the substrate is converted to the product under the reaction condition. Exemplary polypeptides for use in the method, include, but are not limited to, polypeptides comprising the amino acid sequences corresponding to SEQ ID NO: 6, 8, 10, 12, 14, 18, 20, 22, 24, 26, 30, 32, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 64, 66, 68, 70, 74, 76, 78, 80, 82, 86, 88, 90, 92, 104, 106, 110, 112, 126, 130, and 134.

[0278] In some embodiments, a method for reducing the substrate to the product can comprise contacting the substrate with a ketoreductase of the disclosure in a reaction condition comprising at least 100 g/L of substrate and at least 0.8-1.0 g/L of the ketoreductase polypeptide, wherein at least 90% of the substrate is converted to product in 12-20 hrs. In some embodiments, greater than 95%, 96%, 97%, 98%, or 99% or more of the substrate is converted to the product under the reaction condition. Exemplary polypeptides for use in the method, include, but are not limited to, SEQ ID NO: 26, 44, 68, and 104.

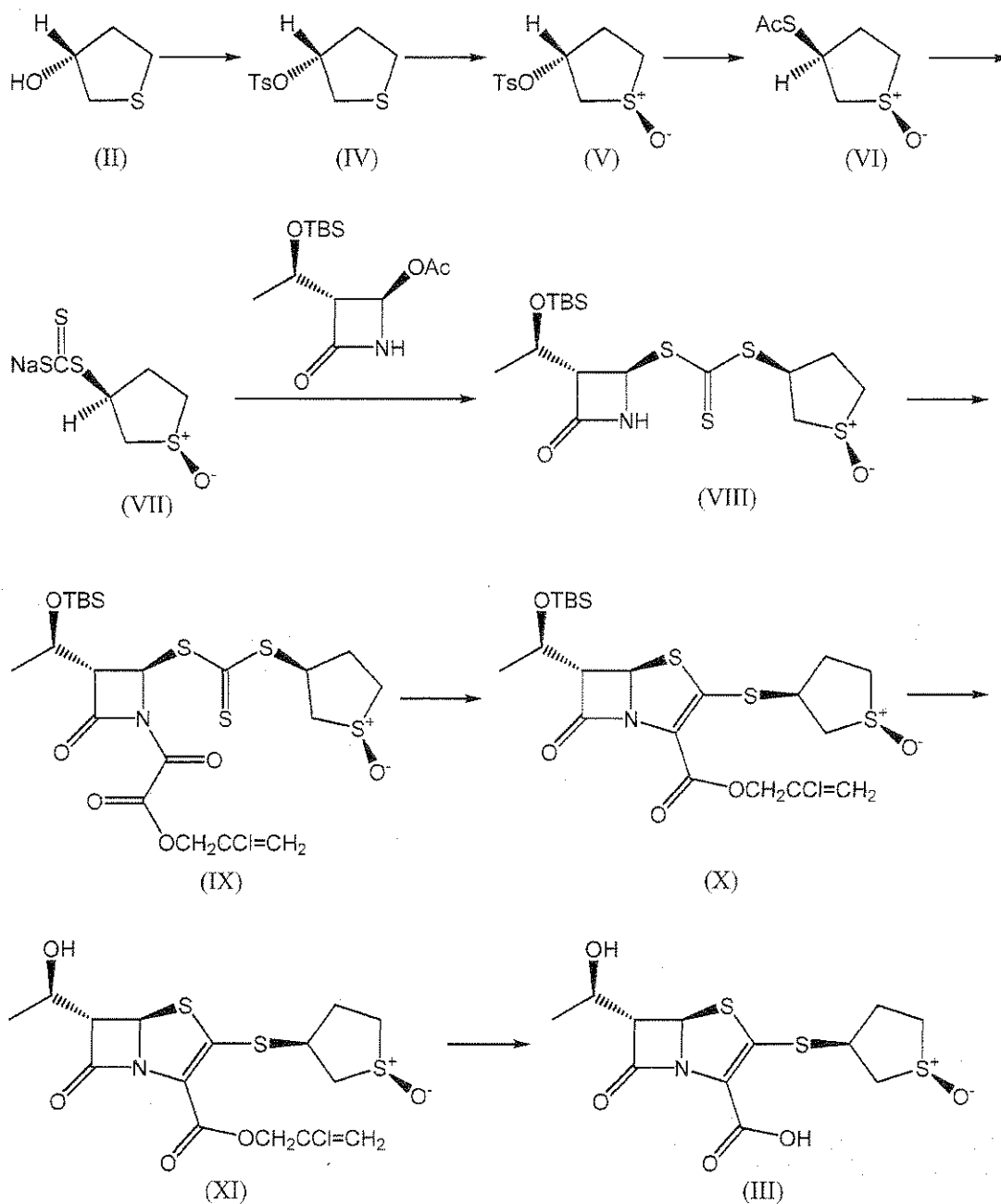
[0279] In some embodiments, any one of the ketoreductases polypeptide provided herein can be used in the production of (*R*)-3-hydroxythiolane, which is an intermediate for producing various drugs, such as antibiotics and protease inhibitors. In some embodiments, the ketoreductase polypeptides can be used in the production of the antibiotic sulopenem (CP-70,429), having the following structural formula (III), and salts thereof, and solvates and hydrates thereof:



[0280] Thus, in some embodiments, in a method for the synthesis of the antibiotic of structural formula (III) (*i.e.*, CP-70,429), a step in the method can comprise contacting the substrate of formula

(I) with any of the ketoreductases described herein, thereby converting or reducing the substrate to the product of structural formula (II).

[0281] The product of structural formula (II) can be converted to sulopenem, having structural formula (III), and intermediates thereto, by the synthetic route described in *J. Org. Chem.*, 1992, 57:4352, incorporated by reference herein. The route described therein is depicted below, wherein Ts is p-tosyl, Ac is acetyl, and TBS is t-butyldimethylsilyl.



[0282] In some embodiments, methods provide for the syntheses of each one of the intermediates of structural formulae (IV), (V), (VI), (VII), (VIII), (IX), (X), and (XI), where a step in the method for synthesis of the intermediates can comprise contacting the substrate of formula (I) with any of the ketoreductases described herein under suitable conditions for converting or reducing the substrate to the product of structural formula (II).

[0283] The additional step or steps in such methods may be according to the procedures for the step or steps described in *J. Org. Chem.*, 1992, 57:4352, incorporated by reference, or modifications thereof. For the conversion of the product of structural formula (II) to the intermediate of structural formula (IV), the dimethylaminopyridine reagent used in the procedure described in *J. Org. Chem.*, 1992, 57:4352 may be substituted with pyridine or 1-methylimidazole. For the conversion of the intermediate of structural formula (IV) to the intermediate of structural formula (V), the reaction that is conducted at 0°C in the procedure described in *J. Org. Chem.*, 1992, 57:4352 may be conducted at lower temperatures, *e.g.*, at -25°C, to further minimize the formation of the corresponding sulfone by-product.

[0284] In some embodiments, the (*R*)-3-hydroxythiolane intermediate can be used in the synthesis of HIV-protease inhibitors (*J. Med. Chem.*, 1994, 37:1177) or other compounds or drugs whose synthesis relies on the intermediate (*R*)-3-hydroxythiolane (formula (II)), wherein a step in the method for synthesis of the drug compound comprises reducing or converting the compound of formula (I) to the compound of formula (II) using any one of the ketoreductase polypeptides provided herein.

[0285] As is known by those of skill in the art, ketoreductase-catalyzed reduction reactions typically require a cofactor. Reduction reactions catalyzed by the engineered ketoreductase enzymes described herein also typically require a cofactor, although many embodiments of the engineered ketoreductases require far less cofactor than reactions catalyzed with wild-type ketoreductase enzymes. As used herein, the term "cofactor" refers to a non-protein compound that operates in combination with a ketoreductase enzyme. Cofactors suitable for use with the engineered ketoreductase enzymes described herein include, but are not limited to, NADP⁺ (nicotinamide adenine dinucleotide phosphate), NADPH (the reduced form of NADP⁺), NAD⁺ (nicotinamide adenine dinucleotide) and NADH (the reduced form of NAD⁺). Generally, the reduced form of the cofactor is added to the reaction mixture. The reduced NAD(P)H form can be optionally regenerated from the oxidized NAD(P)⁺ form using a cofactor regeneration system.

[0286] The term "cofactor regeneration system" refers to a set of reactants that participate in a reaction that reduces the oxidized form of the cofactor (*e.g.*, NADP⁺ to NADPH). Cofactors oxidized

by the ketoreductase-catalyzed reduction of the keto substrate are regenerated in reduced form by the cofactor regeneration system. Cofactor regeneration systems comprise a stoichiometric reductant that is a source of reducing hydrogen equivalents and is capable of reducing the oxidized form of the cofactor. The cofactor regeneration system may further comprise a catalyst, for example an enzyme catalyst, that catalyzes the reduction of the oxidized form of the cofactor by the reductant. Cofactor regeneration systems to regenerate NADH or NADPH from NAD^+ or NADP^+ , respectively, are known in the art and may be used in the methods described herein.

[0287] Suitable exemplary cofactor regeneration systems that may be employed include, but are not limited to, glucose and glucose dehydrogenase, formate and formate dehydrogenase, glucose-6-phosphate and glucose-6-phosphate dehydrogenase, a secondary (*e.g.*, isopropanol) alcohol and secondary alcohol dehydrogenase, phosphite and phosphite dehydrogenase, molecular hydrogen and hydrogenase, and the like. These systems may be used in combination with either $\text{NADP}^+/\text{NADPH}$ or NAD^+/NADH as the cofactor. Electrochemical regeneration using hydrogenase may also be used as a cofactor regeneration system. See, *e.g.*, U.S. Pat. Nos. 5,538,867 and 6,495,023, both of which are incorporated herein by reference. Chemical cofactor regeneration systems comprising a metal catalyst and a reducing agent (for example, molecular hydrogen or formate) are also suitable. See, *e.g.*, PCT publication WO 2000/053731, which is incorporated herein by reference.

[0288] The terms “glucose dehydrogenase” and “GDH” are used interchangeably herein to refer to an NAD^+ or NADP^+ -dependent enzyme that catalyzes the conversion of D-glucose and NAD^+ or NADP^+ to gluconic acid and NADH or NADPH, respectively. Equation (1), below, describes the glucose dehydrogenase-catalyzed reduction of NAD^+ or NADP^+ by glucose.



[0289] Glucose dehydrogenases that are suitable for use in the practice of the methods described herein include both naturally occurring glucose dehydrogenases, as well as non-naturally occurring glucose dehydrogenases. Naturally occurring glucose dehydrogenase encoding genes have been reported in the literature. For example, the *Bacillus subtilis* 61297 GDH gene was expressed in *E. coli* and was reported to exhibit the same physicochemical properties as the enzyme produced in its native host (Vasantha et al., 1983, *Proc. Natl. Acad. Sci. USA* 80:785). The gene sequence of the *B. subtilis* GDH gene, which corresponds to Genbank Acc. No. M12276, was reported by Lampel et al., 1986, *J. Bacteriol.* 166:238-243, and in corrected form by Yamane et al., 1996, *Microbiology* 142:3047-3056 as Genbank Acc. No. D50453. Naturally occurring GDH genes also include those that encode the GDH from *B. cereus* ATCC 14579 (*Nature*, 2003, 423:87-91; Genbank Acc. No.

AE017013) and *B. megaterium* (*Eur. J. Biochem.*, 1988, 174:485-490, Genbank Acc. No. X12370; J. Ferment. Bioeng., 1990, 70:363-369, Genbank Acc. No. GI216270). Glucose dehydrogenases from *Bacillus* sp. are provided in PCT publication WO 2005/018579 as SEQ ID NOS: 10 and 12 (encoded by polynucleotide sequences corresponding to SEQ ID NOS: 9 and 11, respectively, of the PCT publication), the disclosure of which is incorporated herein by reference.

[0290] Non-naturally occurring glucose dehydrogenases may be generated using known methods, such as, for example, mutagenesis, directed evolution, and the like. GDH enzymes having suitable activity, whether naturally occurring or non-naturally occurring, may be readily identified using the assay described in Example 4 of PCT publication WO 2005/018579, the disclosure of which is incorporated herein by reference. Exemplary non-naturally occurring glucose dehydrogenases are provided in PCT publication WO 2005/018579 as SEQ ID NOS: 62, 64, 66, 68, 122, 124, and 126. The polynucleotide sequences that encode them are provided in PCT publication WO 2005/018579 as SEQ ID NOS: 61, 63, 65, 67, 121, 123, and 125, respectively. All of these sequences are incorporated herein by reference. Additional non-naturally occurring glucose dehydrogenases that are suitable for use in the ketoreductase-catalyzed reduction reactions disclosed herein are provided in U.S. application publication Nos. 2005/0095619 and 2005/0153417, the disclosures of which are incorporated herein by reference.

[0291] Glucose dehydrogenases employed in the ketoreductase-catalyzed reduction reactions described herein may exhibit an activity of at least about 10 $\mu\text{mol}/\text{min}/\text{mg}$ and sometimes at least about 10^2 $\mu\text{mol}/\text{min}/\text{mg}$ or about 10^3 $\mu\text{mol}/\text{min}/\text{mg}$, up to about 10^4 $\mu\text{mol}/\text{min}/\text{mg}$ or higher in the assay described in Example 4 of PCT publication WO 2005/018579.

[0292] The ketoreductase-catalyzed reduction reactions described herein are generally carried out in a solvent. Suitable solvents include water, organic solvents (e.g., ethyl acetate, butyl acetate, 1-octanol, heptane, octane, methyl t-butyl ether (MTBE), toluene, and the like), ionic liquids (e.g., 1-ethyl 4-methylimidazolium tetrafluoroborate, 1-butyl-3-methylimidazolium tetrafluoroborate, 1-butyl-3-methylimidazolium hexafluorophosphate, and the like). In some embodiments, aqueous solvents, including water and aqueous co-solvent systems, are used.

[0293] Exemplary aqueous co-solvent systems have water and one or more organic solvent. In general, an organic solvent component of an aqueous co-solvent system is selected such that it does not completely inactivate the ketoreductase enzyme. Appropriate co-solvent systems can be readily identified by measuring the enzymatic activity of the specified engineered ketoreductase enzyme with a defined substrate of interest in the candidate solvent system, utilizing an enzyme activity assay, such as those described herein.

[0294] The organic solvent component of an aqueous co-solvent system may be miscible with the aqueous component, providing a single liquid phase, or may be partly miscible or immiscible with the aqueous component, providing two liquid phases. Generally, when an aqueous co-solvent system is employed, it is selected to be biphasic, with water dispersed in an organic solvent, or vice-versa. Generally, when an aqueous co-solvent system is utilized, it is desirable to select an organic solvent that can be readily separated from the aqueous phase. In general, the ratio of water to organic solvent in the co-solvent system is typically in the range of from about 90:10 to about 10:90 (v/v) organic solvent to water, and between 80:20 and 20:80 (v/v) organic solvent to water. The co-solvent system may be pre-formed prior to addition to the reaction mixture, or it may be formed in situ in the reaction vessel.

[0295] The aqueous solvent (water or aqueous co-solvent system) may be pH-buffered or unbuffered. Generally, the reduction can be carried out at a pH of about 10 or below, usually in the range of from about 5 to about 10. In some embodiments, the reduction is carried out at a pH of about 9 or below, usually in the range of from about 5 to about 9. In some embodiments, the reduction is carried out at a pH of about 8 or below, often in the range of from about 5 to about 8, and usually in the range of from about 6 to about 8. The reduction may also be carried out at a pH of about 7.8 or below, or 7.5 or below. Alternatively, the reduction may be carried out at a neutral pH, *i.e.*, about 7.

[0296] During the course of the reduction reactions, the pH of the reaction mixture may change. The pH of the reaction mixture may be maintained at a desired pH or within a desired pH range by the addition of an acid or a base during the course of the reaction. Alternatively, the pH may be controlled by using an aqueous solvent that comprises a buffer. Suitable buffers to maintain desired pH ranges are known in the art and include, for example, phosphate buffer, triethanolamine buffer, and the like. Combinations of buffering and acid or base addition may also be used.

[0297] When the glucose/glucose dehydrogenase cofactor regeneration system is employed, the co-production of gluconic acid ($pK_a=3.6$), as represented in equation (1) causes the pH of the reaction mixture to drop if the resulting aqueous gluconic acid is not otherwise neutralized. The pH of the reaction mixture may be maintained at the desired level by standard buffering techniques, wherein the buffer neutralizes the gluconic acid up to the buffering capacity provided, or by the addition of a base concurrent with the course of the conversion. Combinations of buffering and base addition may also be used. Suitable buffers to maintain desired pH ranges are described above. Suitable bases for neutralization of gluconic acid are organic bases, for example amines, alkoxides and the like, and inorganic bases, for example, hydroxide salts (*e.g.*, NaOH), carbonate salts (*e.g.*, NaHCO_3), bicarbonate salts (*e.g.*, K_2CO_3), basic phosphate salts (*e.g.*, K_2HPO_4 , Na_3PO_4), and the like. The addition of a base concurrent with the course of the conversion may be done manually while

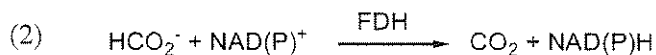
monitoring the reaction mixture pH or, more conveniently, by using an automatic titrator as a pH stat. A combination of partial buffering capacity and base addition can also be used for process control.

[0298] When base addition is employed to neutralize gluconic acid released during a ketoreductase-catalyzed reduction reaction, the progress of the conversion may be monitored by the amount of base added to maintain the pH. Typically, bases added to unbuffered or partially buffered reaction mixtures over the course of the reduction are added in aqueous solutions.

[0299] In some embodiments, the co-factor regenerating system can comprise a formate dehydrogenase. The terms "formate dehydrogenase" and "FDH" are used interchangeably herein to refer to an NAD^+ or NADP^+ -dependent enzyme that catalyzes the conversion of formate and NAD^+ or NADP^+ to carbon dioxide and NADH or NADPH, respectively. Formate dehydrogenases that are suitable for use as cofactor regenerating systems in the ketoreductase-catalyzed reduction reactions described herein include both naturally occurring formate dehydrogenases, as well as non-naturally occurring formate dehydrogenases. Formate dehydrogenases include those corresponding to SEQ ID NOS: 70 (*Pseudomonas* sp.) and 72 (*Candida boidinii*) of PCT publication WO 2005/018579, which are encoded by polynucleotide sequences corresponding to SEQ ID NOS: 69 and 71, respectively, of PCT publication 2005/018579, the disclosures of which are incorporated herein by reference. Formate dehydrogenases employed in the methods described herein, whether naturally occurring or non-naturally occurring, may exhibit an activity of at least about $1 \mu\text{mol/min/mg}$, sometimes at least about $10 \mu\text{mol/min/mg}$, or at least about $10^2 \mu\text{mol/min/mg}$, up to about $10^3 \mu\text{mol/min/mg}$ or higher, and can be readily screened for activity in the assay described in Example 4 of PCT publication WO 2005/018579.

[0300] As used herein, the term "formate" refers to formate anion (HCO_2^-), formic acid (HCO_2H), and mixtures thereof. Formate may be provided in the form of a salt, typically an alkali or ammonium salt (for example, HCO_2Na , KHCO_2NH_4 , and the like), in the form of formic acid, typically aqueous formic acid, or mixtures thereof. Formic acid is a moderate acid. In aqueous solutions within several pH units of its pK_a ($\text{pK}_a=3.7$ in water) formate is present as both HCO_2^- and HCO_2H in equilibrium concentrations. At pH values above about pH 4, formate is predominantly present as HCO_2^- . When formate is provided as formic acid, the reaction mixture is typically buffered or made less acidic by adding a base to provide the desired pH, typically of about pH 5 or above. Suitable bases for neutralization of formic acid include, but are not limited to, organic bases, for example amines, alkoxides and the like; and inorganic bases, for example, hydroxide salts (*e.g.*, NaOH), carbonate salts (*e.g.*, NaHCO_3), bicarbonate salts (*e.g.*, K_2CO_3), basic phosphate salts (*e.g.*, K_2HPO_4 , Na_3PO_4), and the like.

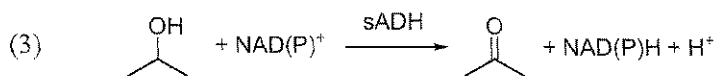
[0301] For pH values above about pH 5, at which formate is predominantly present as HCO_2^- , Equation (2) below, describes the formate dehydrogenase-catalyzed reduction of NAD^+ or NADP^+ by formate.



[0302] When formate and formate dehydrogenase are employed as the cofactor regeneration system, the pH of the reaction mixture may be maintained at the desired level by standard buffering techniques, wherein the buffer releases protons up to the buffering capacity provided, or by the addition of an acid concurrent with the course of the conversion. Suitable acids to add during the course of the reaction to maintain the pH include organic acids, for example carboxylic acids, sulfonic acids, phosphonic acids, and the like, mineral acids, for example hydrohalic acids (such as hydrochloric acid), sulfuric acid, phosphoric acid, and the like, acidic salts, for example dihydrogenphosphate salts (*e.g.*, KH_2PO_4), bisulfate salts (*e.g.*, NaHSO_4) and the like. Some embodiments utilize formic acid, whereby both the formate concentration and the pH of the solution are maintained.

[0303] When acid addition is employed to maintain the pH during a reduction reaction using the formate/formate dehydrogenase cofactor regeneration system, the progress of the conversion may be monitored by the amount of acid added to maintain the pH. Typically, acids added to unbuffered or partially buffered reaction mixtures over the course of conversion are added in aqueous solutions.

[0304] The terms "secondary alcohol dehydrogenase" and "sADH" are used interchangeably herein to refer to an NAD^+ or NADP^+ -dependent enzyme that catalyzes the conversion of a secondary alcohol and NAD^+ or NADP^+ to a ketone and NADH or NADPH , respectively. Equation (3), below, describes the reduction of NAD^+ or NADP^+ by a secondary alcohol, illustrated by isopropanol.



[0305] Secondary alcohol dehydrogenases that are suitable for use as cofactor regenerating systems in the ketoreductase-catalyzed reduction reactions described herein include both naturally occurring secondary alcohol dehydrogenases, as well as non-naturally occurring secondary alcohol dehydrogenases. Naturally occurring secondary alcohol dehydrogenases include known alcohol dehydrogenases from, *Thermoanaerobium brockii*, *Rhodococcus erythropolis*, *Lactobacillus kefir*, and *Lactobacillus brevis*, and non-naturally occurring secondary alcohol dehydrogenases include engineered alcohol dehydrogenases derived therefrom. Secondary alcohol dehydrogenases employed in the methods described herein, whether naturally occurring or non-naturally occurring, may exhibit

an activity of at least about 1 $\mu\text{mol}/\text{min}/\text{mg}$, sometimes at least about 10 $\mu\text{mol}/\text{min}/\text{mg}$, or at least about 10^2 $\mu\text{mol}/\text{min}/\text{mg}$, up to about 10^3 $\mu\text{mol}/\text{min}/\text{mg}$ or higher.

[0306] Suitable secondary alcohols include lower secondary alkanols and aryl-alkyl carbinols. Examples of lower secondary alcohols include isopropanol, 2-butanol, 3-methyl-2-butanol, 2-pentanol, 3-pentanol, 3,3-dimethyl-2-butanol, and the like. In one embodiment the secondary alcohol is isopropanol. Suitable aryl-alkyl carbinols include unsubstituted and substituted 1-arylethanol.

[0307] When a secondary alcohol and secondary alcohol dehydrogenase are employed as the cofactor regeneration system, the resulting NAD^+ or NADP^+ is reduced by the coupled oxidation of the secondary alcohol to the ketone by the secondary alcohol dehydrogenase. Some engineered ketoreductases also have activity to dehydrogenate a secondary alcohol reductant. In some embodiments using secondary alcohol as reductant, the engineered ketoreductase and the secondary alcohol dehydrogenase are the same enzyme.

[0308] In carrying out embodiments of the ketoreductase-catalyzed reduction reactions described herein employing a cofactor regeneration system, either the oxidized or reduced form of the cofactor may be provided initially. As described above, the cofactor regeneration system converts oxidized cofactor to its reduced form, which is then utilized in the reduction of the ketoreductase substrate.

[0309] In some embodiments, cofactor regeneration systems are not used. For reduction reactions carried out without the use of a cofactor regenerating systems, the cofactor is added to the reaction mixture in reduced form.

[0310] In some embodiments, when the process is carried out using whole cells of the host organism, the whole cell may natively provide the cofactor. Alternatively or in combination, the cell may natively or recombinantly provide the glucose dehydrogenase.

[0311] In carrying out the stereoselective reduction reactions described herein, the engineered ketoreductase enzyme, and any enzymes comprising the optional cofactor regeneration system, may be added to the reaction mixture in the form of the purified enzymes, whole cells transformed with gene(s) encoding the enzymes, and/or cell extracts and/or lysates of such cells. The gene(s) encoding the engineered ketoreductase enzyme and the optional cofactor regeneration enzymes can be transformed into host cells separately or together into the same host cell. For example, in some embodiments one set of host cells can be transformed with gene(s) encoding the engineered ketoreductase enzyme and another set can be transformed with gene(s) encoding the cofactor regeneration enzymes. Both sets of transformed cells can be utilized together in the reaction mixture in the form of whole cells, or in the form of lysates or extracts derived therefrom. In other

embodiments, a host cell can be transformed with gene(s) encoding both the engineered ketoreductase enzyme and the cofactor regeneration enzymes.

[0312] Whole cells transformed with gene(s) encoding the engineered ketoreductase enzyme and/or the optional cofactor regeneration enzymes, or cell extracts and/or lysates thereof, may be employed in a variety of different forms, including solid (*e.g.*, lyophilized, spray-dried, and the like) or semisolid (*e.g.*, a crude paste).

[0313] The cell extracts or cell lysates may be partially purified by precipitation (ammonium sulfate, polyethyleneimine, heat treatment or the like, followed by a desalting procedure prior to lyophilization (*e.g.*, ultrafiltration, dialysis, and the like). Any of the cell preparations may be stabilized by crosslinking using known crosslinking agents, such as, for example, glutaraldehyde or immobilization to a solid phase (*e.g.*, Eupergit C, and the like).

[0314] The solid reactants (*e.g.*, enzyme, salts, etc.) may be provided to the reaction in a variety of different forms, including powder (*e.g.*, lyophilized, spray dried, and the like), solution, emulsion, suspension, and the like. The reactants can be readily lyophilized or spray dried using methods and equipment that are known to those having ordinary skill in the art. For example, the protein solution can be frozen at -80°C in small aliquots, then added to a prechilled lyophilization chamber, followed by the application of a vacuum. After the removal of water from the samples, the temperature is typically raised to 4°C for two hours before release of the vacuum and retrieval of the lyophilized samples.

[0315] The quantities of reactants used in the reduction reaction will generally vary depending on the quantities of product desired, and concomitantly the amount of ketoreductase substrate employed. The following guidelines can be used to determine the amounts of ketoreductase, cofactor, and optional cofactor regeneration system to use. Generally, keto substrates can be employed at a concentration of about 20 to 300 grams/liter using from about 50 mg to about 5 g of ketoreductase and about 10 mg to about 150 mg of cofactor. Those having ordinary skill in the art will readily understand how to vary these quantities to tailor them to the desired level of productivity and scale of production. Appropriate quantities of optional cofactor regeneration system may be readily determined by routine experimentation based on the amount of cofactor and/or ketoreductase utilized. In general, the reductant (*e.g.*, glucose, formate, isopropanol) is utilized at levels above the equimolar level of ketoreductase substrate to achieve essentially complete or near complete conversion of the ketoreductase substrate.

[0316] The order of addition of reactants is not critical. The reactants may be added together at the same time to a solvent (*e.g.*, monophasic solvent, biphasic aqueous co-solvent system, and the like),

or alternatively, some of the reactants may be added separately, and some together at different time points. For example, the cofactor regeneration system, cofactor, ketoreductase, and ketoreductase substrate may be added first to the solvent.

[0317] For improved mixing efficiency when an aqueous co-solvent system is used, the cofactor regeneration system, ketoreductase, and cofactor may be added and mixed into the aqueous phase first. The organic phase may then be added and mixed in, followed by addition of the ketoreductase substrate. Alternatively, the ketoreductase substrate may be premixed in the organic phase, prior to addition to the aqueous phase

[0318] Suitable conditions for carrying out the ketoreductase-catalyzed reduction reactions described herein include a wide variety of conditions which can be readily optimized by routine experimentation that includes, but is not limited to, contacting the engineered ketoreductase enzyme and substrate at an experimental pH and temperature and detecting product, for example, using the methods described in the Examples provided herein.

[0319] The ketoreductase catalyzed reduction is typically carried out at a temperature in the range of from about 15°C to about 75°C. For some embodiments, the reaction is carried out at a temperature in the range of from about 20°C to about 55°C. In still other embodiments, it is carried out at a temperature in the range of from about 20°C to about 45°C. The reaction may also be carried out under ambient conditions.

[0320] The reduction reaction is generally allowed to proceed until essentially complete, or near complete, reduction of substrate is obtained. Reduction of substrate to product can be monitored using known methods by detecting substrate and/or product. Suitable methods include gas chromatography, HPLC, and the like. Conversion yields of the alcohol reduction product generated in the reaction mixture are generally greater than about 50%, may also be greater than about 60%, may also be greater than about 70%, may also be greater than about 80%, may also be greater than 90%, and are often greater than about 97%.

8. EXAMPLES

[0321] Various features and embodiments of the disclosure are illustrated in the following representative examples, which are intended to be illustrative, and not limiting.

8.1 Example 1: Wild-type Ketoreductase Gene Acquisition and Construction of Expression Vectors.

[0322] Ketoreductase (KRED) encoding genes were designed for expression in *E. coli* based on the reported amino acid sequence of the ketoreductase and a codon optimization algorithm as described in

Example 1 of US provisional application Serial No. 60/848,950 and WO2008042876, incorporated herein by reference. Genes were synthesized using oligonucleotides composed of 42 nucleotides and cloned into expression vector pCK110900 (depicted as FIG. 3 in United States Patent Application Publication 20060195947) under the control of a lac promoter. The expression vector also contained the P15a origin of replication and the chloramphenicol resistance gene. Resulting plasmids were transformed into *E. coli* W3110 using standard methods. Sequences of codon optimized genes and the encoded polypeptides as well are listed in Table 3. The activity of the wild-type ketoreductases was confirmed as described in US provisional application Serial No. 60/848,950.

Table 3: Abbreviations, source and references for Ketoreductases used					
KRED	Microorganism from which enzyme was originally identified	Genbank Acc. No.	GI no.	Polynucleotide SEQ ID No	Polypeptide SEQ ID No, Or Source
ADH-CM	<i>Candida magnoliae</i>	AB036927.1	12657576	SEQ ID No 1 in US Patent Appln Publn 20060195947	SEQ ID No 2 in US Patent Appln Publn 20060195947
YDL	<i>Saccharomyces cerevisiae</i>	NP_010159.1	6320079	SEQ ID NO: 146	SEQ ID NO:147
ADH-LB	<i>Lactobacillus brevis</i>	1NXQ_A	30749782	SEQ ID NO:1 (codon optimized)	SEQ ID NO:2
ADH-RE	<i>Rhodococcus erythropolis</i>	AAN73270.1	34776951	SEQ ID NO:148	SEQ ID NO:149
YGL	<i>Saccharomyces cerevisiae</i>	NP_011476	6321399	SEQ ID NO:150	SEQ ID NO:151
YPR	<i>Saccharomyces cerevisiae</i>	NP_010656.1	6320576	SEQ ID NO:152	SEQ ID NO:153
GRE	<i>Saccharomyces cerevisiae</i>	NP_014490.1	6324421	SEQ ID NO:154	SEQ ID NO:155
ADH-LK	<i>Lactobacillus kefir</i>	AAP94029.1	33112056	SEQ ID NO:3 (codon optimized)	SEQ ID NO:4
ADH-SB	<i>Sporobolomyces salmonicolor</i>	Q9UUN9	30315955	SEQ ID NO:137	SEQ ID NO:138

ADH-SC	<i>Streptomyces coelicolor</i>	NP_631415.1	21225636	SEQ ID NO:135	SEQ ID NO:136
ADH-TB	<i>Thermoanaerobium Brockii</i>	X64841.1	1771790	SEQ ID NO:139	SEQ ID NO:140
ADH-CP	<i>Candida parapsilosis</i>	BAA24528	2815409		Julich Chiral Solutions No. 03.11
DR-LB	<i>Lactobacillus brevis</i> (diacetyl reductase)	ABJ63353.1	116098204		Julich Chiral Solutions No. 8.1

[0323] Polynucleotides encoding engineered ketoreductases of the present invention were likewise cloned into vector pCK110900 for expression in *E. coli* W3110.

8.2 Example 2: Production of Ketoreductase Powders; Shake Flask Procedure.

[0324] A single microbial colony of *E. coli* containing a plasmid with the ketoreductase gene of interest was inoculated into 50 ml Luria Bertani broth containing 30 µg/ml chloramphenicol and 1% glucose. Cells were grown overnight (at least 16 hrs) in an incubator at 30°C with shaking at 250 rpm. The culture was diluted into 250 ml Terrific Broth (12 g/L bacto-tryptone, 24g/L yeast extract, 4 ml/L glycerol, 65mM potassium phosphate, pH 7.0, 1mM MgSO₄, 30 µg/ml chloramphenicol) in 1 liter flask) to an optical density at 600 nm (OD₆₀₀) of 0.2 and allowed to grow at 30°C. Expression of the ketoreductase gene was induced with 1mM IPTG when the OD₆₀₀ of the culture is 0.6 to 0.8 and incubated overnight (at least 16 hrs). Cells were harvested by centrifugation (5000rpm, 15 min, 4°C) and the supernatant discarded. The cell pellet was resuspended with an equal volume of cold (4°C) 100mM triethanolamine (chloride) buffer, pH 7.0 (including 2 mM MgSO₄ in the case of ADH-LK and ADH-LB and engineered ketoreductases derived therefrom), and harvested by centrifugation as above. The washed cells were resuspended in two volumes of the cold triethanolamine (chloride) buffer and passed through a French Press twice at 12000psi while maintained at 4°C. Cell debris was removed by centrifugation (9000 rpm, 45 min., 4°C). The clear lysate supernatant was collected and stored at -20°C. Lyophilization of frozen clear lysate provided a dry powder of crude ketoreductase enzyme.

[0325] The activity of the wild-type ketoreductases was confirmed as described US provisional application Serial No. 60/848,950. To a solution of 1 mL 100mM (sodium) phosphate buffer, pH 7.5, were added 10 mg ketoreductase powder, 50 mg NAD(P)H, 100 µL isopropanol and 10 mg

4'-chloroacetophenone or unsubstituted acetophenone. The reaction mixture was stirred at room temperature for 16 hours, then extracted with 1 mL MTBE. A sample of the MTBE phase was analyzed by chiral HPLC for the conversion of the 4'-chloro-acetophenone and the enantiomeric composition of the product.

8.3 Example 3: Production of Ketoreductases - Fermentation Procedure.

[0326] In an aerated agitated 15L fermenter, 6.0L of growth medium containing 0.88 g/L ammonium sulfate, 0.98 g/L of sodium citrate, 12.5 g/L of dipotassium hydrogen phosphate trihydrate, 6.25g/L of potassium dihydrogen phosphate, 6.2 g/L of Tastone-154 yeast extract, 0.083 g/L ferric ammonium citrate, and 8.3 ml/L of a trace element solution containing 2 g/L of calcium chloride dihydrate, 2.2 g/L of zinc sulfate septahydrate, 0.5 g/L manganese sulfate monohydrate, 1 g/L cuprous sulfate heptahydrate, 0.1 g/L ammonium molybdate tetrahydrate and 0.02 g/L sodium tetraborate decahydrate was brought to a temperature of 30°C. The fermenter was inoculated with a late exponential culture of *E. coli* W3110, containing a plasmid with the ketoreductase gene of interest, grown in a shake flask as described in Example 2 to a starting OD600 of 0.5 to 2.0. The fermenter was agitated at 500-1500 rpm and air was supplied to the fermentation vessel at 1.0-15.0 L/min to maintain dissolved oxygen level of 30% saturation or greater. The pH of the culture was controlled at 7.0 by addition of 20% v/v ammonium hydroxide. Growth of the culture was maintained by the addition of a feed solution containing 500g/L cerelese, 12 g/L ammonium chloride and 10.4 g/L magnesium sulfate heptahydrate. After the culture reached an OD600 of 50, the expression of ketoreductase was induced by the addition of isopropyl- β -D-thiogalactoside (IPTG) to a final concentration of 1mM. The culture was grown for another 14 hours. The culture was then chilled to 4°C and maintained at 4°C until harvested. Cells were harvested by centrifugation at 5000G for 40 minutes in a Sorval RC12BP centrifuge at 4°C. Harvested cells were used directly in the following downstream recovery process or were stored at 4°C until such use.

[0327] The cell pellet was resuspended in 2 volumes of 100 mM triethanolamine (chloride) buffer, pH 6.8, at 4°C to each volume of wet cell paste. The intracellular ketoreductase was released from the cells by passing the suspension through a homogenizer fitted with a two-stage homogenizing valve assembly using a pressure of 12000 psig. The cell homogenate was cooled to 4°C immediately after disruption. A solution of 10% w/v polyethyleneimine, pH 7.2, was added to the lysate to a final concentration of 0.5% w/v and stirred for 30 minutes. The resulting suspension was clarified by centrifugation at 5000G in a standard laboratory centrifuge for 30 minutes. The clear supernatant was decanted and concentrated ten fold using a cellulose ultrafiltration membrane with a molecular weight cut off of 30Kd. The final concentrate was dispensed into shallow containers, frozen at -20°C and

lyophilized to powder. The ketoreductase powder was stored at -80°C .

8.4 Example 4: Analytical methods to determine conversion of 3-ketothiolane and enantiomeric excess of R-3-hydroxythiolane.

[0328] Achiral GC to determine conversion: Reduction of 3-ketothiolane to 3-hydroxythiolane was determined using an Agilent HP-5MS Gas chromatograph equipped with a 5% phenyl methyl siloxane column (Model 19091S-433: 30 m x 250 μm , nominal thickness 0.25 μm) with a helium flow rate of 1.0 mL/min. The inlet temperature was 220°C and the following temperature program was used: 120°C for 1 min then $20^{\circ}\text{C}/\text{min}$ to 160°C then 160°C for 0.5 min. Compounds were detected spectrophotometrically at 210 nm. The retention times of the ketone and alcohol were 2.8 minutes, and 3.0 minutes respectively.

[0329] Chiral HPLC to determine stereomeric purity of 3-hydroxythiolane: The abundance of the R and S enantiomers of 3-hydroxythiolane was determined using an Agilent 1100 or 1050 HPLC equipped with a Chiralpak AD column (4.6 x 250 mm and no guard column) with IPA/hexane (2/98) as eluent at a flow rate of 2.5 mL/min at room temperature. The retention times of the ketone, S-alcohol and R-alcohol were 8.6, 12.0 and 13.1 minute respectively

[0330] Alternatively, an HPLC equipped with a Chiralpak AD-H column (4.6 x 150 mm and 10 mm guard column) was used with IPA/hexane (2/98) at a flow rate of 2.0 mL/min at 40°C . Compounds were detected spectrophotometrically at 210 nm. The retention times of the ketone, S-alcohol and R-alcohol were 6.3, 8.7 and 9.4 minute respectively.

8.5 Example 5: Evaluation of Wild-Type Ketoreductases for Reduction of 3-ketothiolane.

[0331] The ketoreductases described in Table 3 (Example 1) were screened for enantioselective reduction of 3-ketothiolane under co-factor limiting conditions: to a vial under air was added 1-20 mg of enzyme, 1 mL of a co-factor stock solution containing 766 mg of NAD(P)H dissolved in 20 mL of 100 mM pH 8.0 triethanolamine(chloride) buffer and 20 μL 3-ketothiolane. After stirring at room temperature overnight, the reaction mixture was extracted with ethyl acetate and the enantioselectivity was assayed as described in Example 4. Results are described in Table 4. (NR= no reaction.)

Table 4: Activities of wild-type KREDs on 3-ketothiolane					
Source	Co-Factor Used		e.e. (%)	Configuration	
	NADH	NADPH		R	S
<i>GRE</i>		X	48		X
<i>YPR</i>		X	42		X
<i>YGL</i>		X	11	X	
<i>ADH-LB</i>		X	46	X	
ADH-LK		X	67	X	
<i>ADH-SB</i>		X	66		X
<i>ADH-SC</i>		X	NR		
YDL		X	~0		
YDL	X		~0		
<i>ADH-RE</i>	X		~70		X
ADH-RE		X	~70		X
<i>ADH-CP</i>		X	NR		
<i>ADH-CP</i>	X		~ 80		X
<i>ADH-TB</i>		X	57		X
DR-LB		X	10	X	
DR-LB	X		12	X	

[0332] This example shows that ADH-LK is the most R-enantioselective ketoreductase among the wild-type enzymes tested, giving R-3-hydroxythiolane in 67% e.e. Accordingly, ADH-LK was chosen as starting point for enzyme engineering towards the desired characteristics.

8.6 Example 6: High Throughput NADPH Fluorescence Prescreen To Identify Improved Enzymes for the Reduction of 3-Ketothiolane.

[0333] Plasmid libraries containing evolved ketoreductase genes were transformed into *E. coli* W3110 and plated on Luria-Bertani (LB) agar plates containing 1% glucose and 30 µg/mL chloramphenicol (CAM). After incubation for at least 16 hrs at 30°C, colonies were picked using a Q-bot® robotic colony picker (Genetix USA, Inc., Beaverton, OR) into shallow, 96-well microtiter plates containing 180 µL LB, 1% glucose and 30 µg/mL CAM. Cells were grown overnight at 37°C with shaking at 250 rpm. 10 µL of this culture was then transferred into 96-well microtiter plates (deep well) containing 390 µL Terrific broth (TB) and 30 µg/mL CAM. After incubation of deep-well plates at 30°C with shaking at 250 rpm for 2.5 to 3 hours (OD_{600} 0.6-0.8), recombinant gene expression by the cell cultures was induced by isopropyl thiogalactoside (IPTG) to a final concentration of 1 mM. The plates were then incubated at 30°C with shaking at 250 rpm for overnight.

[0334] Cells were pelleted via centrifugation, resuspended in 300 µL lysis buffer and lysed by shaking at room temperature for at least 2 hours. The lysis buffer contained 100 mM triethanolamine (chloride) buffer, pH 7.0-7.2, 1 mg/mL lysozyme and 750 µg/mL polymixin B sulfate. The plates were centrifuged at 4000RPM for 10 minutes and the clear supernatant (lysate) used in the fluorescent assay.

[0335] In 96-well black microtiter plates 20 µL of clear supernatant (diluted in 100 mM triethanolamine/chloride buffer, pH 7.0, 1mM $MgSO_4$ if necessary) was added to 180 µL of an assay mixture consisting of 100 mM triethanolamine(chloride) buffer, pH 7.0, 1mM $MgSO_4$, 0.25 mM NADPH, 600 mM glucose, 1200 mM sodium gluconate and 1 mM 3-ketothiolane and reaction progress measured by following the decrease in fluorescence of NADPH at 445 nm after excitation at 330 nm in a Flexstation (Molecular Devices, USA). To assess thermostability of KRED variants, clear supernatants were optionally pre-incubated for at least 16 hrs at a temperature ranging from 40 to 50°C, and then added into the assay mixture.

[0336] This example describes the method that was used to identify KRED variants improved for the rate of 3-ketothiolane reduction and/or improved for thermostability.

8.7 Example 7: High Throughput HPLC Assay for Ketoreductase Activity and Enantioselectivity for the Reduction of 3-Ketothiolane using Glucose/Glucose Dehydrogenase for Co-factor Recycling.

[0337] Lysates were prepared as described in Example 6. Ketoreductase activity was measured by transferring measured quantities of the cell lysates into the wells of deep well microtiter plates. The assay mixture in the wells (final volume 500 μ L each well) contained 1 mg/mL GDH, 0.4 mg/mL NADP, 0.2 to 1.25 M glucose 0 to 400 mM sodium gluconate, 100 mM triethanolamine(chloride) buffer (pH 7), 20 mM $MgSO_4$ and up to 50 mg calcium carbonate. Reactions were initiated by addition of up to 100 μ L cell lysate and 50 μ L 3-ketothiolane (final concentration 1M). After sealing with aluminum/polypropylene laminate heat seal tape (Velocity 11 (Menlo Park, CA), Cat# 06643-001), the plates were incubated at 4 to 25°C for up to 16 hrs. 1 mL ethyl acetate was injected into each well of the plates and the plates were shaken for 10 minutes, then centrifuged for 20 seconds; 100 μ L of the ethyl acetate phase was transferred to shallow well microtiter plates. These sample plates were sealed with heat seal tape to prevent evaporation. The samples were analyzed by HPLC by the method of Example 4.

[0338] This example shows how ketoreductase variants with improved activity for the reduction of 3-ketothiolane and/or improved enantioselectivity for the formation of R-3-hydroxythiolane were identified in ketoreductase gene libraries.

8.8 Example 8: Reduction of 3-Ketothiolane by Engineered Ketoreductases Derived from ADH-LK.

[0339] Improved ketoreductases derived from ADH-LK variants were evaluated at preparative scale as follows. To a 25 mL three-neck vessel equipped with a PTFE-coated magnetic stirring bar and a pH electrode connected to an automatic titrator for pH-controlled addition of base on-demand via a feeding tube into the vessel, was charged with 18 mL aqueous (water or buffer) phase containing 6.63 g glucose. Subsequently, 3.0 g 3-ketothiolane (re-distilled), 30 to 120 mg KRED variant in 1 mL of the aqueous phase, 30 mg GDH in 1 mL of the aqueous phase, and 12 mg NADP- Na_2 were added. The automatic titrator maintained the pH at 7 by the addition of 4N NaOH, which was continuously recorded. Reaction progress was monitored by the rate and cumulative addition of the base and periodic sampling of the reaction mixture for extraction with ethyl acetate and analysis by the method of Example 4. The concentration of R-3-hydroxythiolane at reaction completion was 100 $\pm$ 5 g/L.

[0340] Table 5 gives the SEQ ID NOs. corresponding to ketoreductase variants, the number of amino acid mutations from the wild-type ADH-LK, their activities for reduction of 3-ketothiolane (as amounts of enzyme powder required to completely convert the 3-ketothiolane to 3-hydroxythiolane in a 24 hr reaction), and their R-enantioselectivities for the 3-hydroxythiolane product (as e.e. ranges).

[0341] This Example illustrates that engineered ketoreductases derived from the ketoreductase ADH-LK that provide improved enantioselectivities and activities compared to the wild-type ketoreductase ADH-LK.

Table 5: Improved activities and stabilities of engineered ADH-LK variants				
SEQ ID NO.	mutations from ADH-LK	Enantioselectivity ^a	Activity ^b	Stability ^c
4	none	0	0	0
120	1	0	0	0
166	1	0	0	0
122	2	0	0	0
114	2	+	0	0
118	3	+	0	0
132	6	+	0	0
84	3	+	0	0
98	1	++	0	0
124	6	0	++	+
102	2	+++	0	0
90	2	+++	+	+
16	3	+++	+	0
100	2	+++	0	0
108	2	+++	0	0
128	3	+++	0	0
96	2	+++	0	0
130	2	+++	+	0
126	2	+++	+	0

104	5	+++	++	+
88	3	+++	+	+
106	6	+++	+	+
14	5	+++	+	+
86	6	+++	+	+
44	6	+++	++	+
92	4	+++	+	+
112	8	+++	+	+
94	4	+++	0	-
12	4	+++	+	+
56	8	+++	+	+
110	6	+++	+	+
36	7	++++	0	+
46	7	+++	+	+
68	8	+++	++	+
48	8	+++	+	-
74	7	+++	+	+
82	6	+++	+	+
42	9	++++	+	+
24	8	++++	+	+
34	8	++++	0	+
38	8	++++	+	+
32	8	+++	+	+
8	8	++++	+	+
134	8	++++	+	+

6	8	++++	+	+
26	9	+++++	++	+
72	10	+++++	0	-
18	10	++++	+	0
20	9	++++	+	0
54	9	++++	+	0
10	10	++++	+	0
40	9	++++	+	0
52	7	++++	+	+
50	8	++++	+	+
76	9	++++	+	+
80	10	+++++	+	+
78	10	++++	+	+
30	10	+++++	+	+
28	10	+++++	0	0
22	10	+++++	+	+
60	9	+++	+	+
64	9	+++	+	+
70	9	++++	+	+
62	10	++++	0	0
66	10	++++	+	+
58	11	+++++	+	+

^a Enantioselectivity column:

0 61.0-79.99%ee for the (*R*) enantiomer

+ 80.0-89.99%ee for the (*R*) enantiomer

- ++ 90.0-94.99%ee for the (*R*) enantiomer
- +++ 95.0-97.99%ee for the (*R*) enantiomer
- ++++ 98.0-98.99%ee for the (*R*) enantiomer
- +++++ >99.0%ee for the (*R*) enantiomer

^b Activity column:

- 0 A concentration of 1.0-4.0 g/L of this enzyme is required to give full conversion of 100 g/L 3-ketothiolane within 24 h
- + A concentration of 0.8-1.0 g/L of this enzyme gives full conversion of 100 g/L 3-ketothiolane in 20 to 24 h
- ++ A concentration of 0.8-1.0 g/L of this enzyme gives full conversion of 100 g/L 3-ketothiolane in 12 to 20 h

^c Stability column:

- less stable than ADH-LK
- 0 similarly stable as ADH-LK
- + more stable than ADH-LK

8.9 Example 9: Preparative scale production of R-(3)-hydroxythiolane.

[0342] To a 2-L three-neck jacketed flask under air at room temperature and fitted with mechanical stirrer at ~300 rpm, a thermometer, a pH electrode connected to an automatic titrator for pH-controlled addition of base on-demand via a feeding tube into the vessel, was added 600 mL 100 mM pH 7.0 triethanolamine(chloride) buffer, 2 mL 1 M MgSO₄, and 225 g D-glucose. After dissolution of the glucose (~½ h), 100 g 3-ketothiolane was added to the reaction to give a biphasic mixture. The reaction temperature was lowered to 15°C and 1.0 g ketoreductase powder (SEQ-ID No. 22) in 30 mL 100 mM pH 7.0 triethanolamine(chloride) buffer and 0.25 g GDH in 20 mL 100 mM pH 7.0 triethanolamine(chloride) buffer, and 0.4 g of NADP-Na₂ in 10 mL 100 mM pH 7.0 triethanolamine(chloride) buffer was added. The reaction temperature was maintained at 15°C via an external circulating chiller and the automatic titrator maintained the pH at 7.0±0.1 by the addition of 8N NaOH. After stirring at 15°C for 15 h the circulator temperature was raised to 25°C and after 1 additional hour, 0.25 g GDH powder was added (final GDH concentration=0.5 g/L). After a total of

23 hours, 10 g $\text{Na}_2\text{S}_2\text{O}_5$ was added. After stirring for one additional hour at 25°C , the product was extracted with 750 mL ethyl acetate. The separated organic phase was filtered through ~20 g Celite 545. The wet Celite pad was rinsed with 750 mL EtOAc. The combined organic phases were washed with 100 mL water. The separated organic phase was rotatory evaporated under vacuum at 60°C for give 83.6 g (82% yield) of 3-hydroxythiolane as a pale yellow oil. Chiral HPLC analysis showed the (*R*)-3-hydroxythiolane in 99.3% enantiomeric excess.

[0343] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes.

[0344] While various specific embodiments have been illustrated and described, it will be appreciated that various changes can be made without departing from the spirit and scope of the invention(s).

What is claimed is:

1. A ketoreductase polypeptide capable of stereoselectively reducing a 3-ketothiolane to (R)-3-hydroxythiolane with a percent stereomeric excess of at least 70%, which comprises an amino acid sequence that is at least 85% identical to a reference sequence based on SEQ ID NO:2 or 4 or 142 having at the residue corresponding to X145 a serine, and in which the ketoreductase polypeptide amino acid sequence has at the residue corresponding to X145 a serine.

2. The polypeptide of claim 1 in which the ketoreductase polypeptide amino acid sequence has one or more features selected from the following:

- residue corresponding to X3 is a polar, acidic, or aromatic residue;
- residue corresponding to X7 is a non-polar, polar, or constrained residue;
- residue corresponding to X11 is an aliphatic, non-polar, or polar residue;
- residue corresponding to X16 is an aliphatic or non-polar residue;
- residue corresponding to X19 is a non-polar or aliphatic residue;
- residue corresponding to X23 is a non-polar or aromatic residue;
- residue corresponding to X41 is an aliphatic, non-polar, or polar residue;
- residue corresponding to X45 is an aliphatic, non-polar, or polar residue;
- residue corresponding to X49 is a basic residue;
- residue corresponding to X57 is an aliphatic or non-polar residue;
- residue corresponding to X60 is an aromatic, aliphatic, non-polar, or polar residue;
- residue corresponding to X64 is an aliphatic or non-polar residue;
- residue corresponding to X72 is a basic residue;
- residue corresponding to X82 is a non-polar or polar residue;
- residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue;
- residue corresponding to X95 is a non-polar or aliphatic residue;
- residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue;
- residue corresponding to X97 is acidic, basic, or aliphatic residue;
- residue corresponding to X106 is an acidic residue;
- residue corresponding to X108 is a basic, constrained, or aromatic residue;

residue corresponding to X111 is an aliphatic or non-polar residue;
 residue corresponding to X117 is a non-polar or polar residue;
 residue corresponding to X126 is an aliphatic or non-polar residue;
 residue corresponding to X127 is a polar or basic residue;
 residue corresponding to X147 is an aromatic, aliphatic residue, non-polar, or polar residue;
 residue corresponding to X152 is a polar, aliphatic or non-polar residue;
 residue corresponding to X157 is a polar or acidic residue;
 residue corresponding to X163 is an aliphatic or non-polar residue;
 residue corresponding to X173 is an acidic or non-polar residue;
 residue corresponding to X177 is a basic residue;
 residue corresponding to X192 is a basic or acidic residue;
 residue corresponding to X194 is a constrained, polar, non-polar, acidic, or aliphatic residue;
 residue corresponding to X198 is an acidic or non-polar residue;
 residue corresponding to X200 is a constrained, acidic, or basic residue;
 residue corresponding to X206 is a polar or non-polar residue;
 residue corresponding to X208 is a polar, constrained, or basic residue;
 residue corresponding to X210 is an aliphatic, non-polar, or polar residue;
 residue corresponding to X211 is a basic or acidic residue;
 residue corresponding to X214 is a non-polar, aliphatic or polar residue;
 residue corresponding to X217 is an aromatic, aliphatic, or non-polar residue;
 residue corresponding to X223 is an aliphatic or non-polar residue;
 residue corresponding to X226 is a non-polar or aliphatic residue; and
 wherein the amino acid sequence can optionally have one or more differences at other amino acid residues as compared to the reference sequence.

3. The polypeptide of claim 1 in which the ketoreductase polypeptide amino acid sequence has one or more features selected from the following:

residue corresponding to X3 is asparagine, aspartic acid, or tyrosine;
 residue corresponding to X7 is glycine, histidine, serine or asparagine;
 residue corresponding to X11 is isoleucine or threonine;
 residue corresponding to X16 is threonine, alanine, valine, or glycine;
 residue corresponding to X19 is isoleucine or valine;
 residue corresponding to X23 is isoleucine or phenylalanine;
 residue corresponding to X41 is serine, alanine, or valine;
 residue corresponding to X45 is glutamic acid or glycine;

residue corresponding to X49 is lysine or arginine;
residue corresponding to X57 is isoleucine or valine;
residue corresponding to X60 is phenylalanine, valine, or threonine;
residue corresponding to X64 is alanine, serine, or threonine;
residue corresponding to X72 is lysine or arginine, particularly arginine;
residue corresponding to X82 is glycine or serine;
residue corresponding to X94 is alanine, valine, threonine, serine, or arginine;
residue corresponding to X95 is valine or alanine;
residue corresponding to X96 is asparagine, serine, proline, alanine, or glutamic acid;
residue corresponding to X97 is lysine, arginine or leucine;
residue corresponding to X106 is glutamic acid or aspartic acid;
residue corresponding to X108 is arginine or histidine;
residue corresponding to X111 is leucine or methionine;
residue corresponding to X117 is glycine or serine;
residue corresponding to X126 is isoleucine or valine;
residue corresponding to X127 is glutamine or arginine;
residue corresponding to X147 is phenylalanine, leucine or serine;
residue corresponding to X152 is threonine, serine, or methionine;
residue corresponding to X157 is asparagine, glutamine, threonine, serine, or aspartic acid;
residue corresponding to X163 is valine or isoleucine;
residue corresponding to X173 is aspartic acid or glycine;
residue corresponding to X177 is lysine or arginine;
residue corresponding to X192 is lysine, arginine or glutamic acid;
residue corresponding to X194 is proline, glycine, aspartic acid, arginine, or leucine;
residue corresponding to X198 is aspartic acid or glycine;
residue corresponding to X200 is proline, glutamic acid, or lysine;
residue corresponding to X206 is methionine or glutamine;
residue corresponding to X208 is glutamine, histidine or arginine;
residue corresponding to X210 is threonine or alanine;
residue corresponding to X211 is lysine or glutamic acid;
residue corresponding to X214 is methionine, valine or threonine, or serine;
residue corresponding to X217 is isoleucine or phenylalanine;
residue corresponding to X223 is isoleucine or valine;
residue corresponding to X226 is isoleucine or valine; and

wherein the amino acid sequence can optionally have one or more differences at other amino acid residues as compared to the reference sequence.

4. The polypeptide of claim 1 in which the ketoreductase polypeptide amino acid sequence has one or more of the features selected from the following:

- residue corresponding to X7 is a non-polar, polar, or constrained residue;
- residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue;
- residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue;
- residue corresponding to X108 is a basic, constrained, or aromatic residue;
- residue corresponding to X117 is a non-polar or polar residue;
- residue corresponding to X157 is a polar or acidic residue;
- residue corresponding to X173 is an acidic or non-polar residue;
- residue corresponding to X206 is a polar or non-polar residue;
- residue corresponding to X223 is an aliphatic or non-polar residue; and

wherein the amino acid sequence can optionally have one or more differences at other amino acid residues as compared to the reference sequence.

5. The polypeptide of claim 1 in which the ketoreductase polypeptide amino acid sequence has at least the following features;

- residue corresponding to X117 is a non-polar or polar residue;
- residue corresponding to X157 is a polar or acidic residue; and

wherein the amino acid sequence can optionally have one or more differences at other amino acid residues as compared to the reference sequence.

6. The polypeptide of claim 1 in which the ketoreductase polypeptide amino acid sequence has at least the following features:

- residue corresponding to X7 is a non-polar, polar, or constrained residue;
- residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue;
- residue corresponding to X108 is a basic, constrained, or aromatic residue;
- residue corresponding to X117 is a non-polar or polar residue, particularly serine;
- residue corresponding to X157 is a polar or acidic residue, particularly a threonine; and

wherein the amino acid sequence can optionally have one or more differences at other amino acid residues as compared to the reference sequence.

7. The polypeptide of claim 1 in which the ketoreductase polypeptide amino acid sequence has at least the following features:

- residue corresponding to X7 is a non-polar, polar, or constrained residue;
- residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue;
- residue corresponding to X108 is a basic, constrained, or aromatic residue;
- residue corresponding to X117 is a non-polar or polar residue, particularly serine;
- residue corresponding to X157 is a polar or acidic residue, particularly a threonine; and
- wherein the amino acid sequence can optionally have one or more differences at other amino acid residues as compared to the reference sequence.

8. The polypeptide of claim 1 in which the ketoreductase polypeptide amino acid sequence has at least the following features:

- residue corresponding to X7 is a non-polar, polar, or constrained residue;
- residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue;
- residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue;
- residue corresponding to X108 is a basic, constrained, or constrained residue;
- residue corresponding to X117 is a non-polar or polar residue, particularly serine;
- residue corresponding to X157 is a polar or acidic residue, particularly a threonine; and
- wherein the amino acid sequence can optionally have one or more differences at other amino acid residues as compared to the reference sequence.

9. The polypeptide of claim 1 in which the ketoreductase polypeptide amino acid sequence has at least the following features:

- residue corresponding to X7 is a non-polar, polar, or constrained residue;
- residue corresponding to X108 is a basic, constrained, or aromatic residue;
- residue corresponding to X117 is a non-polar or polar residue;
- residue corresponding to X157 is a polar or acidic residue;
- residue corresponding to X206 is a polar or non-polar residue; and
- wherein the amino acid sequence can optionally have one or more differences at other amino acid residues as compared to the reference sequence.

10. The polypeptide of claim 1 in which the ketoreductase polypeptide amino acid sequence has at least the following features:

- residue corresponding to X7 is a non-polar, polar, or constrained residue;
- residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue;

residue corresponding to X108 is a basic, constrained, or aromatic residue;
residue corresponding to X117 is a non-polar or polar residue;
residue corresponding to X157 is a polar or acidic residue;
residue corresponding to X206 is a polar or non-polar residue; and
wherein the amino acid sequence can optionally have one or more differences at other amino acid residues as compared to the reference sequence.

11. The polypeptide of claim 1 in which the ketoreductase polypeptide amino acid sequence has at least the following features:

residue corresponding to X7 is a non-polar, polar, or constrained residue;
residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue;
residue corresponding to X108 is a basic, constrained, or aromatic residue;
residue corresponding to X117 is a non-polar or polar residue;
residue corresponding to X157 is a polar or acidic residue;
residue corresponding to X206 is a polar or non-polar residue; and
wherein the amino acid sequence can optionally have one or more differences at other amino acid residues as compared to the reference sequence.

12. The polypeptide of claim 1 in which the ketoreductase polypeptide amino acid sequence has at least the following features:

residue corresponding to X7 is a non-polar, polar, or constrained residue;
residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue;
residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue;
residue corresponding to X108 is a basic, constrained, or aromatic residue;
residue corresponding to X117 is a non-polar or polar residue;
residue corresponding to X157 is a polar or acidic residue;
residue corresponding to X206 is a polar or non-polar residue; and
wherein the amino acid sequence can optionally have one or more differences at other amino acid residues as compared to the reference sequence.

13. The polypeptide of claim 1 in which the ketoreductase polypeptide amino acid sequence has at least the following features:

residue corresponding to X117 is a non-polar or polar residue;
residue corresponding to X145 is a polar residue;
residue corresponding to X157 is a polar or acidic residue;

residue corresponding to X173 is an acidic or non-polar residue;
residue corresponding to X206 is a polar or non-polar residue; and
wherein the amino acid sequence can optionally have one or more differences at other amino acid residues as compared to the reference sequence.

14. The polypeptide of claim 1 in which the ketoreductase polypeptide amino acid sequence has at least the following features:

residue corresponding to X7 is a non-polar, polar, or constrained residue;
residue corresponding to X117 is a non-polar or polar residue;
residue corresponding to X145 is a polar residue;
residue corresponding to X157 is a polar or acidic residue;
residue corresponding to X173 is an acidic or non-polar residue;
residue corresponding to X206 is a polar or non-polar residue; and
wherein the amino acid sequence can optionally have one or more differences at other amino acid residues as compared to the reference sequence.

15. The polypeptide of claim 1 in which the ketoreductase polypeptide amino acid sequence has at least the following features:

residue corresponding to X7 is a non-polar, polar, or constrained residue;
residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue;
residue corresponding to X117 is a non-polar or polar residue;
residue corresponding to X145 is a polar residue;
residue corresponding to X157 is a polar or acidic residue;
residue corresponding to X173 is an acidic or non-polar residue;
residue corresponding to X206 is a polar or non-polar residue; and
wherein the amino acid sequence can optionally have one or more differences at other amino acid residues as compared to the reference sequence.

16. The polypeptide of claim 1 in which the ketoreductase polypeptide amino acid sequence has at least the following features:

residue corresponding to X7 is a non-polar, polar, or constrained residue;
residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue;
residue corresponding to X117 is a non-polar or polar residue;
residue corresponding to X145 is a polar residue;
residue corresponding to X157 is a polar or acidic residue;

residue corresponding to X173 is an acidic or non-polar residue;
residue corresponding to X206 is a polar or non-polar residue; and
wherein the amino acid sequence can optionally have one or more differences at other amino acid residues as compared to the reference sequence.

17. The polypeptide of claim 1 in which the ketoreductase polypeptide amino acid sequence has at least the following features:

residue corresponding to X7 is a non-polar, polar, or constrained residue;
residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue;
residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue;
residue corresponding to X117 is a non-polar or polar residue;
residue corresponding to X145 is a polar residue;
residue corresponding to X157 is a polar or acidic residue;
residue corresponding to X173 is an acidic or non-polar residue;
residue corresponding to X206 is a polar or non-polar residue; and
wherein the amino acid sequence can optionally have one or more differences at other amino acid residues as compared to the reference sequence.

18. The polypeptide of claim 1, which comprises a domain having an amino acid sequence that is at least 85% identical to residues 90 to 211 of a reference sequence based on SEQ ID NO:2 or 4 or 142.

19. The polypeptide of claim 18, wherein the amino acid sequence that corresponds to residues 90 to 211 has one or more of the features selected from:

residue corresponding to X94 is a polar, basic, aliphatic, or non-polar residue;
residue corresponding to X95 is a nonpolar or aromatic residue;
residue corresponding to X96 is a constrained, aliphatic, non-polar, acidic, or polar residue;
residue corresponding to X106 is an acidic residue
residue corresponding to X108 is a basic, constrained, or aromatic residue;
residue corresponding to X111 is an aliphatic or non-polar residue;

residue corresponding to X117 is a non-polar or polar residue;
 residue corresponding to X126 is an aliphatic or non-polar residue;
 residue corresponding to X127 is a polar or basic residue;
 residue corresponding to X147 is an aromatic, aliphatic residue, non-polar, or polar residue;
 residue corresponding to X152 is a polar, aliphatic or non-polar residue;
 residue corresponding to X157 is a polar or acidic residue;
 residue corresponding to X163 is an aliphatic or non-polar residue;
 residue corresponding to X173 is an acidic or non-polar residue;
 residue corresponding to X177 is a basic residue;
 residue corresponding to X192 is a basic or acidic residue;
 residue corresponding to X194 is a constrained, polar, non-polar, acidic, or aliphatic residue;
 residue corresponding to X198 is an acidic or non-polar residue;
 residue corresponding to X200 is a constrained, acidic, or basic residue;
 residue corresponding to X206 is a polar or non-polar residue;
 residue corresponding to X208 is a polar, constrained, or basic residue;
 residue corresponding to X210 is an aliphatic, non-polar, or polar residue;
 residue corresponding to X211 is a basic or acidic residue; and
 wherein the amino acid sequence can optionally have one or more differences at other amino acid residues in the domain corresponding to residues 90-211 as compared to the reference sequence.

20. The polypeptide of claim 18, wherein the amino acid sequence that corresponds to residues 90 to 211 has one or more of the features selected from:

residue corresponding to X94 is alanine, valine, threonine, serine, or arginine;
 residue corresponding to X95 is valine or alanine;
 residue corresponding to X96 is asparagine, serine, proline, alanine, or glutamic acid;
 residue corresponding to X97 is lysine, arginine, or leucine;
 residue corresponding to X106 is glutamic acid or aspartic acid;
 residue corresponding to X108 is arginine or histidine;
 residue corresponding to X111 is leucine or methionine;
 residue corresponding to X117 is glycine or serine;
 residue corresponding to X126 is isoleucine or valine;
 residue corresponding to X127 is glutamine or arginine;
 residue corresponding to X147 is phenylalanine, leucine or serine;
 residue corresponding to X152 is threonine, serine, or methionine;
 residue corresponding to X157 is asparagine, threonine, serine, or aspartic acid;

residue corresponding to X163 is valine or isoleucine;
residue corresponding to X173 is aspartic acid or glycine;
residue corresponding to X177 is lysine or arginine;
residue corresponding to X192 is lysine, arginine or glutamic acid;
residue corresponding to X194 is proline, glycine, aspartic acid, arginine, or leucine;
residue corresponding to X198 is aspartic acid or glycine;
residue corresponding to X200 is proline, glutamic acid, or lysine;
residue corresponding to X206 is methionine or glutamine;
residue corresponding to X208 is glutamine, histidine or arginine;
residue corresponding to X210 is threonine or alanine;
residue corresponding to X211 is lysine or glutamic acid; and
wherein the amino acid sequence can optionally have one or more differences at other amino acid residues in the domain corresponding to residues 90-211 as compared to the reference sequence..

21. The polypeptide of claim 1, which comprises a domain having an amino acid sequence that is at least 85% identical to residues 1 to 89 of a reference sequence based on SEQ ID NO:2 or 4 or 142 having at the residue corresponding to residue X7 a serine.

22. The polypeptide of claim 21 in which the domain can have additionally one or more features selected from the following.

residue corresponding to X3 is a polar, acidic, or aromatic residue;
residue corresponding to X11 is an aliphatic, non-polar, or polar residue;
residue corresponding to X16 is an aliphatic or non-polar residue;
residue corresponding to X19 is a non-polar or aliphatic residue;
residue corresponding to X23 is a non-polar or aromatic residue;
residue corresponding to X41 is an aliphatic, non-polar, or polar residue;
residue corresponding to X57 is an aliphatic or non-polar residue;
residue corresponding to X60 is an aromatic, aliphatic, non-polar, or polar residue;
residue corresponding to X72 is a basic residue;
residue corresponding to X82 is a non-polar or polar residue; and
wherein the amino acid sequence can optionally have one or more differences at other amino acid residues in the domain corresponding to residues 1-89 as compared to the reference sequence..

23. The polypeptide of claim 21, wherein the amino acid sequence that corresponds to residues 1 to 89 has one or more of the following features selected from:

- residue corresponding to X3 is asparagine, aspartic acid, or tyrosine;
- residue corresponding to X11 is isoleucine or threonine;
- residue corresponding to X16 is threonine, alanine, valine, or glycine;
- residue corresponding to X19 is isoleucine or valine;
- residue corresponding to X23 is isoleucine or phenylalanine;
- residue corresponding to X41 is serine, alanine, or valine;
- residue corresponding to X49 is lysine or arginine;
- residue corresponding to X57 is isoleucine or valine;
- residue corresponding to X60 is phenylalanine, valine, or threonine;
- residue corresponding to X72 is lysine or arginine; and
- residue corresponding to X82 is glycine or serine; and

wherein the amino acid sequence can optionally have one or more differences at other amino acid residues in the domain corresponding to residues 1-89 as compared to the reference sequence.

24. The polypeptide of claim 1, which comprises a domain having an amino acid sequence that is at least 85% identical to residues 212 to 252 of a reference sequence based on SEQ ID NO:2 or 4 or 142 having at the residue corresponding to residue X223 a valine.

25. The polypeptide of claim 24, wherein the amino acid sequence that corresponds to residues 212 to 252 has one or more of the following features selected from:

- residue corresponding to X214 is a non-polar, aliphatic or polar residue;
- residue corresponding to X217 is an aromatic, aliphatic, or non-polar residue;
- residue corresponding to X223 is an aliphatic or non-polar residue;
- residue corresponding to X226 is a non-polar or aliphatic residue and

wherein the amino acid sequence can optionally have one or more differences at other amino acid residues in the domain corresponding to residues 212-252 as compared to the reference sequence.

26. The polypeptide of claim 24, wherein the amino acid sequence that corresponds to residues 212 to 252 has one or more of the following features selected from:

- residue corresponding to X214 is a methionine, valine, threonine, or serine;
- residue corresponding to X217 is an isoleucine or phenylalanine;
- residue corresponding to X223 is an isoleucine or valine;

residue corresponding to X226 is an isoleucine or valine; and

wherein the amino acid sequence can optionally have one or more differences at other amino acid residues in the domain corresponding to residues 212-252 as compared to the reference sequence.

27. The polypeptide of claim 1, wherein the percent stereomeric excess is at least 90%, wherein the polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 86, 88, 90, 92, 94, 96, 100, 102, 104, 106, 108, 110, 112, 126, 128, 130, or 134.

28. The polypeptide of claim 1, wherein the percent stereomeric excess is at least 98%, wherein the polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 6, 8, 10, 18, 20, 22, 24, 26, 28, 30, 34, 36, 38, 40, 42, 50, 52, 54, 58, 62, 66, 70, 72, 76, 78, 80, or 134.

29. The polypeptide of claim 1, which is capable of converting 3-ketothiolane to (R)-3-hydroxythiolane at a rate that is improved over the wild-type polypeptide of SEQ ID NO:4, wherein the polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 64, 66, 68, 70, 74, 76, 78, 80, 86, 88, 90, 92, 104, 106, 110, 112, 126, 130, or 134.

30. The polypeptide of claim 1, which is capable of stereoselectively reducing 3-ketothiolane to (R)-3-hydroxythiolane with a percent stereomeric excess of at least 95%, which has improved activity and thermostability as compared to the wild-type polypeptide of SEQ ID NO:4, and comprises an amino acid sequence corresponding to SEQ ID NO: 6, 8, 12, 14, 22, 24, 26, 30, 32, 38, 42, 44, 46, 50, 52, 56, 58, 60, 64, 66, 68, 70, 74, 76, 78, 80, 82, 86, 88, 90, 92, 104, 106, 110, 112, or 134.

31. A polynucleotide encoding a polypeptide according to any one of claims 1 to 30.

32. The polynucleotide of claim 31 which is selected from the group consisting of SEQ ID NO: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 125, 127, 129, and 133.

33. An expression vector comprising the polynucleotide of claim 31 or 32 operably linked to at least one control sequence suitable for directing expression in a host cell.

34. The expression vector of claim 33, wherein the control sequence comprises a promoter.

35. The expression vector of claim 34, wherein the promoter comprises an E. coli promoter.

36. The expression vector of claim 33, wherein the control sequence comprises a secretion signal.

37. A host cell comprising the expression vector of claim 33.

38. The host cell of claim 37, which is E. coli.

39. The host cell of claim 37, wherein the codons comprising the expression vector have been optimized for expression in the host cell.

40. A method for stereoselectively reducing substrate 3-ketothiolane to product (R)-3-hydroxythiolane, which comprises contacting the 3-ketothiolane with the ketoreductase polypeptide of any one of claims 1 to 30 under suitable reactions conditions for reducing or converting the 3-ketothiolase to the (R)-3-hydroxythiolane.

41. The method of claim 40, wherein 3-ketothiolane is reduced to (R)-3-hydroxythiolane with at least 70% stereomeric excess.

42. The method of claim 40, wherein 3-ketothiolane is reduced to (R)-3-hydroxythiolane with at least 90% stereomeric excess with the ketoreductase polypeptide.

43. The method of claim 40, wherein the 3-ketothiolase is reduced to (R)-3-hydroxythiolane with at least 98% stereomeric excess.

44. The method of claim 40, wherein the substrate is at least 100 g/L and at least 90% of the substrate is converted to product in less than 24 hrs, wherein the ketoreductase polypeptide is at about 0.8-1.0 g/L and is selected from the group consisting of SEQ ID NO: 6, 8, 10, 12, 14, 18, 20, 22, 24, 26, 30, 32, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 64, 66, 68, 70, 74, 76, 78, 80, 82, 86, 88, 90, 92, 104, 106, 110, 112, 126, 130, and 134.

45. The method of claim 40, wherein the substrate is at least 100 g/L and at least 90% of the substrate is converted to product in less than 20 hrs, wherein the ketoreductase polypeptide is at about 0.8-1.0 g/L and is selected from the group consisting of SEQ ID NO: 26, 44, 68, and 104.

46. The method of claim 40 which is carried out at a pH of about 7.5 or less.

47. The method of claim 40 which is carried at a pH of from about 5.0 to about 7.5.

48. The method of claim 40 which is carried out with whole cells that express the ketoreductase enzyme, or an extract or lysate of such cells.

49. The method of claim 40 in which the ketoreductase is isolated and/or purified and the reduction reaction is carried out in the presence of a cofactor for the ketoreductase and optionally a regeneration system for the cofactor.

50. A composition comprising the compound 3-ketothiolane and a ketoreductase of claim 1.

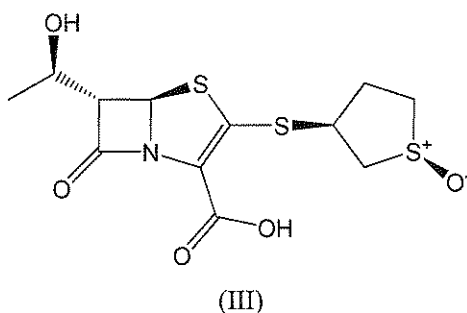
51. The composition of claim 50, further comprising a cofactor regenerating system.

52. The method of claim 49 in which the cofactor regenerating system comprises glucose dehydrogenase and glucose; formate dehydrogenase and formate; or isopropanol and a secondary alcohol dehydrogenase.

53. The method of claim 52 wherein the secondary alcohol dehydrogenase is the ketoreductase.

54. The composition of claim 51 in which the cofactor regenerating system comprises glucose dehydrogenase and glucose; formate dehydrogenase and formate; or isopropanol and a secondary alcohol dehydrogenase.

55. The method of claim 40, wherein the method is for the synthesis of the antibiotic of structural formula (III).



SEQUENCE LISTING

<110> LIANG, Jack
JENNE, Stephane J.
MUNDORFF, Emily
VOLADRI, Rama
LALONDE, James
HUISMAN, Gjaat

<120> IMPROVED KETOREDUCTASE POLYPEPTIDES FOR THE STEREOSELECTIVE PRODUCTION OF (R)-3-HYDROXYTHIOLANE

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<151> 2007-08-24

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Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
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376247016

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 <223> Variant of L. kefir

<400> 8
 Met Thr Asp Arg Leu Lys Asn Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Asp Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 9
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 9
 atgaccgatac gtctgaagag caaagtagcc atcgtaacgg gcgggacctt gggtatcggt 60

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tiggcaatcg cggataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gcggaigtg gtaaaaaggc cgccaaatca atcgccggta ctgatgttat tgcctttgc 180
 cagcagatg catccgatg agcaggctgg acgaaactgt tcgacaccac caggaggca 240
 ttgcgcccgg ttacgaccgt cgtgaacaat gcagggatta ccgtttcaa aagcgttgaa 300
 gacactacca cggaggaatg gcacaaactg ctgtccgta atctggatag tgtttttc 360
 ggcacccgtc tgggcatca gcgcatgaag aataaaggct tggcgctag catcatcaat 420
 atgagcagta tcatgggtt cgtaggcgt cgcagctgg gggcatacac tgcctcaag 480
 ggggcggtag gtatcatgtc gaaaagcgc gcgctggatt gcgcactgag ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgctggtoga tgaatcggaa 600
 ggtgctgagg aaatgcaatc acggcgtagc aaaaccctta tgggccacat tggcgaaccg 660
 aatgacgtgg catggaatc tgtgtacctg gcactgacg aatcgaaatt tgcgaagggt 720
 gcagaatttg tggcgaaggc cgggtatacc gcacagtga 759

<210> 10
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 10
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Arg Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Gln Ser Arg
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 11
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 11
 atgaccgatc gtctgaagag caaagtagcc atcgtaacgc gcgggactct gggatccgtt 60
 tiggcaatcg cggataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gcggaigtg gtaaaaaggc cgccaaatca atcgccggca ctgatgttat tgcctttgc 180
 cagcagatg catccgatg agcaggctgg acgaaactgt tcgacaccac caggaggca 240
 ttgcgcccgg ttacgaccgt cgtgaacaat gcagggattg cagtttcaa aagcgttgaa 300
 gacactacca cggaggaatg gcgtaaactg ctgtccgta atctggatag tgtttttc 360
 ggcacccgtc tgggcatca gcgcatgaaa aataaaggct tggcgctag catcatcaat 420
 atgagcagta tcatgggtt cgtaggcgt cgcagctgg gggcatacac tgcctcaag 480
 ggggcggtag gtatcatgtc gaaaagcgc gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgctggtoga tgaatcggaa 600

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gggtgctgagg aaatgatgtc acagcgtacg aaaaccoccta tgggccacat tggcgaaccg 660
aatgacatcg catggatctg tgtgtacctg gcattctgacg aatcgaaaatt tgcgacgggt 720
gcagaatttg tggcgcacgg cgggtatacc gcacagtga 759

<210> 12
<211> 252
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 12
Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
1 5 10 15
Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
20 25 30
Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
35 40 45
Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
50 55 60
Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
65 70 75 80
Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
85 90 95
Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
100 105 110
Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
115 120 125
Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
130 135 140
Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
145 150 155 160
Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
165 170 175
Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
180 185 190
Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
195 200 205
Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
210 215 220
Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
225 230 235 240
Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
245 250

<210> 13
<211> 759
<212> DNA
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 13
atgaccgatc gtctgaagag caaagtagcc atcgtaacgg gcgggactct gggtatcggg 60
ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggtcgccac 120
gcggatgtag gtgaaaaggc cgcacaaatca atcgccggca ctgatgttat tcgcttgtc 180
cagcacgaig catccgatga agcaggctgg acgaaactgt tgcacaccac cgaggaggca 240
ttcggcccggt ttaacgacct cgtgaacaat gcaggggattg cagtttccaa aagcgttgaa 300
gacactacca cggagggaatg gcacaaactg ctgtccggtta atctggatag tgttttttc 360
ggcaccgcgc tgggcattca gcgcataaaa aataaaggct tgggcgctag catcatcaat 420
atgagcagta tcagtgggtt cgtaggcgat ccgacgctgg gggcatacac tgcttccaag 480
ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgctggtcga tgaatcggaa 600
gggtgctgagg aaatgatgtc acagcgtacg aaaaccoccta tgggccacat tggcgaaccg 660
aatgacatcg catggatctg tgtgtacctg gcattctgacg aatcgaaaatt tgcgacgggt 720
gcagaatttg tggcgcacgg cgggtatacc gcacagtga 759

<210> 14
<211> 252
<212> PRT
<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 14

Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 15

<211> 759

<212> DNA

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 15

atgaccgac gtctgaagag caaagtagcc atcgtaaccg gcgggactct gggtatcggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gcggatgtag gtgaaaaggc cgcaaatca atcgcgggca ctgatgttat tgcgtttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgaccgt cgtgaacaat gcagggattg cagtttcaa aagcgttgaa 300
 gacactacca cggaggaatg gcgtaaactg ctgtccgita atctggatgg tgttttttc 360
 ggcacccgtc tgggcattca gcgcatgaaa aataaaggct tggcgctag catcatcaat 420
 atgagcagta tcagtgggtt cgtaggcgat ccgacgctgg gggcatacaa cgcttccaag 480
 ggggcggtag gtatcatgtc gaaaagcgca gcgctggatt gcgactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tcggggtat atcaagacct cgtagtcca tgatctggaa 600
 ggtgctgagg aaatgatgtc acagcgtacg aaaacccta tgggccacat tggcgaaacc 660
 aatgacgtcg catggatctg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 16

<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 16

Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30

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Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Asn Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 17
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 17
 atgaccgatac gtctgaagag caaagtagcc atcgtaaccg gcgggactct gggatccggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gcggaigtac gtgaaaaggc cgccaaatca atcgcggtta ctgatgtat tgccttctc 180
 cagcacgacg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgaccgt cgtgaacaat gcagggatta ccgttccaaa aagcgtigaa 300
 gacactacca cggaggaatg gcacaaactg ctgtccgtta atctggatag tgttttttc 360
 ggcaccgctc tgggcattca gcgcatgaag aataaaggct tggcgctag catcatcaat 420
 atgagcagta tcagtgggtc cgttggcgat ccgacgctgg gggcatacac tgcctccaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt ggcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc tgcgtgtcga tcatctggaa 600
 ggtgctgagg aatgatgtc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
 aatgacgtgg catggtatct tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 18
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 18
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Pro
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser

100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Leu Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Leu Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 19
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 19
 atgaccgac gtcgaagag caaagtagcc atcgtaaccg gcgggactct gggtatcgg 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcgcggtga ctgatgtat tcgcttgtc 180
 cagcacgata catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgaccgt cgtgaacaat gcagggatta cgttcctaaa aagcgttgaa 300
 gacactacca cggagggaatg gcacaaactg ctgtccgtta atctggatag tgttttttc 360
 ggcaccgcgc tgggcattca gcgcatgaag aataaaggct tggcgctag catcatcaat 420
 atgagcagta tcagtggtt cgtaggcgt cgcagcgtgg ggcatacac tgcctcaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgtggtcga tcatcggaa 600
 ggtgctgagg aaatgatgtc acagcgtacg aaaaccctta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatcgt tgtgtacctg gcactgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tgtcgacgg cgggtatacc gcacagtga 759

<210> 20
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 20
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Thr
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Pro
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175

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Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gin
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gin
 245 250

<210> 21
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 21
 atgaccgatac gtcgaagag caaagtagcc atcgtaaccc gcgggactct gggtatcggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaag tagttattac tggcgccac 120
 gcggatgtag gtgaaaaggc cgcaaatca atcgccggtta ctgatttat tgccttctc 180
 cagcacgatg catccgatga agcaggctgg acgaaacigt tgcacaccac cgaggaggca 240
 ttccggccgg ttacgaccgt cgtgaacaat gcagggatta cgttccaaa aagcgtgaa 300
 gacactacca cggaggaatg gcacaaactg ctgtccgta atctggatag tgtttttc 360
 ggcaccgcgc tgggcaatca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtgggtt cgtaggcgat ccgacgctgg gggcatacac tgcctccaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagacca acctggctga tgatctggaa 600
 ggtgctgagg aaatgcaatc acagcgtagc aaaacccta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatcgt tgtgtacctg gcactgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 22
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 22
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Pro
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Asn Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Gln Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gin

245

250

<210> 23
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 23
 atgaccgatac gtctgaagag caaagtagcc atcgtaaccg gcgggactct gggtatcggg 60
 ttggcaatcg ccgataaalt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcgccggta ctgatgtat tcgctttgc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttcgcccggt ttacgaccgt cggaacaat gcagggatta ccgttccaaa aagcgtgaa 300
 gacactacca cggaggaatg gcacaaactg ctgtccgta atctggatag tgtttttc 360
 ggcaccgcgtc tgggcattca gcgcatgaag aataaaggct tggcgctag catcatcaat 420
 atgagcagta tcagtgggtt cgtaggcgt cgcacgctgg gggcatacac tgctccaag 480
 gggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagacct cgtcgtcga tgatctggaa 600
 ggtgctgagg aaatgatgtc acagcgtacg aaaaccctta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatctg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cggtataacc gcacagtga 759

<210> 24
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 24
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Pro
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 25
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 25

atgaccgatac gtcgaagag caaagtagcc atcgtaacgc gcgggactct gggtatcggt 60
 ttggcaatcg cggataaatt ttagaggag ggtgcgaag tagttattac tggcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcggcggta ctgatgtat tcgcttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgaccgt cgtgaacaat gcagggatta ccgttccaaa aagcgttgaa 300
 gacactacca cggaggaatg gcacaaactg ctgtccgtta atctggatag tgttttttc 360
 ggcaccgcgc tgggcattca gcgcataag aataaaggct tgggcgctag catcalcaat 420
 atgagcagta tcagtgggtt ctagggcgat ccgacgctgg gggcatacac tgctccaag 480
 ggggcggtag gtatcatgic gaaaagcgca gcgctggatt gcgcactgaa ggactacat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgtgtgtcga tgatctggaa 600
 ggtgtcgtgagg aaatgcaatc acagcgtacg aaaaccctta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatctg tgtgtacctg gcactgtacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacggc cgggtatacc gcacagtga 759

<210> 26

<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 26

Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Pro
 85 90 95
 Lys Ser Val Glu Asp Ser Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Gln Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 27

<211> 759

<212> DNA

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 27

atgaccgatac gtcgaagag caaagtagcc atcgtaacgc gcgggactct gggtatcggt 60
 ttggcaatcg cggataaatt ttagaggag ggtgcgaag tagttattac tggcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcggcggta ctgatgtat tcgcttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgaccgt cgtgaacaat gcagggatta ccgttccaaa aagcgttgaa 300
 gacactacca cggaggaatg gcacaaactg ctgtccgtta atctggatag tgttttttc 360

376247016

ggcaccgcgc tgggcattca ggcgatgaag aataaaggct tgggcgctag catcatcaat 420
atgagcagta tcagtgggtt cgtaggcgat ccgacgctgg gggcatacac tgctccaag 480
ggggcggtac gtatcatgic gaaaagcgca ggcctggatt ggcactgaa ggactacgat 540
gtgcgtgtca acacagtaca tccgggctat atcaagaccg acctgggtga tgatctggaa 600
gggtctgagg aaatgcaatc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
aatgacgtgg catggtatcg tgtgtacctg gcactgacg aatcgaaatt tgcgacgggt 720
gcagaatttg tggtcgacgg cgggtatacc gcacagtga 759

<210> 28
<211> 252
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 28
Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
1 5 10 15
Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
20 25 30
Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
35 40 45
Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
50 55 60
Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
65 70 75 80
Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Pro
85 90 95
Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
100 105 110
Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
115 120 125
Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
130 135 140
Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
145 150 155 160
Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
165 170 175
Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
180 185 190
Thr Asp Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Gln Ser Gln
195 200 205
Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
210 215 220
Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
225 230 235 240
Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
245 250

<210> 29
<211> 759
<212> DNA
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 29
atgaccgacg gtcgaagag caaagtagcc atcgtaaccg gcgggactct gggatcggt 60
ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggtcgccac 120
gcggatgtag gtgaaaaggc cgccaaatca atcgccggta ctgatgtat tgcctttgc 180
cagcagcatg catccgaiga agcaggctgg acgaaactgt tgcacaccac cgaggaggca 240
ttcgcccggt itacgacgtg cgtgaacaat gcagggatta ccgttccaaa aagcgttgaa 300
gacactacca cggagggaatg gcacaaactg ctgtccgtta atctggatag tgttttttc 360
ggcaccgcgc tgggcattca ggcgatgaag aataaaggct tgggcgctag catcatcaat 420
atgagcagta tcagtgggtt cgtaggcgat ccgacgctgg gggcatacac tgctccaag 480
ggggcggtac gtatcatgic gaaaagcgca ggcctggatt ggcactgaa ggactacgat 540
gtgcgtgtca acacagtaca tccgggctat atcaagaccg ggcctgggtga tgatctggaa 600
gggtctgagg aaatgcaatc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
aatgacgtgg catggtatcg tgtgtacctg gcactgacg aatcgaaatt tgcgacgggt 720
gcagaatttg tggtcgacgg cgggtatacc gcacagtga 759

<210> 30
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 30
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Pro
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Gly Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Gln Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 31
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 31
 atgaccgac gtctgaagag caaagtagcc atcgtaaccg gcgggactct gggtatcggt 60
 ttggcaatcg cggataaatt ttagaggag ggtagcgaag tagttattac tggcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcgccggta ctgatgttat tcgcttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttccggcccg ttacgacgt cgtgaacaat gcagggatta ccgttgagaa aagcgttgaa 300
 gacactacca cggaggaatg gcacaaactg ctgtccgtta atctggatag tgttttttc 360
 ggcaccgcgc tgggcattca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtgggtt cgtaggcgat ccgacgctgg gggcatacac tgcttccaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgttggtcga tgatctggaa 600
 ggtgctgagg aaatgatgtc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatctg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgcacgg cgggtatacc gcacagtga 759

<210> 32
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 32

376247016

Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Glu
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 33
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 33
 atgaccgatac gtctgaagag caaagtagcc atcgtaacgc gcgggactct gggtatcggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggtcgccac 120
 gcggatgtag gtgaaaaggc cgcacaatca atcggcggta ctgatgtat tcgctttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac caggaggca 240
 ttggcccggt ttacgaccgt cgtgaacaat gcagggatta ccgttgccaa aagcgtgaa 300
 gacactacca cggaggaatg gcacaaactg ctgtcgtta atctggatag tgttttttc 360
 ggcacccgtc tgggcattca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcatgggtt cgtaggcgat ccgacgcgg gggcatacac tgctccaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgtcgttga tgatctggaa 600
 ggtgctgagg aaatgatgtc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatctg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 34
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 34
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala

65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Ala
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 35
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 35
 atgaccgatac gtcgaagag caaagtagcc atcgtaaccg gcgggactct gggtatcggf 60
 ttggcaatgc ccgataaatt tctagaggag ggtgcgaaag tagttattac tggtcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcgccggtg ctgattttat tgcctttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgacgtt cgtgaacaat gcaggggatta cgtttccaa aagcgttgaa 300
 gacactacca cggagggaatg gcacaaactg ctgtccgtta atctggatag tgttttttc 360
 ggcaccgcgc tgggcattca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtgggtt cgtaggcgat ccgacgctgg gggcatacac tgcctccaag 480
 ggggcggtac gtaicgtgc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgctggctga tgatctggaa 600
 ggtgctgagg aatatgtgc acagcgtacg aaaaccctta tgggccacat tggcgaaccg 660
 aatgacgtgg catggtatctg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggctgacgg cgggtatacc gcacagtga 759

<210> 36
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 36
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140

376247016

Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 37
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 37
 atgaccgatac gtctgaagag caaagtagcc atcgtaaccg gcgggactct gggtatcggt 60
 ttggcaatcg cggataaatt ttagaggag ggtcgaaag tagttattac tggcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcggcggtg ctgatgtat tgccttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttccggccgg ttacgacgti cgtgaacaat gcagggatta ccgtttccaa aagcgttgaa 300
 gacactacca cggagggaatg gcacaaactg ctgtccgtta atctggatag tgttttttc 360
 ggcacccgtc tgggcattca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtggtt cgtaggcgat ccgacgctgg gggcatcac tgcctccaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgctgggtcga tgatctggaa 600
 gggtctgagg aaatgcaatc acagcgtacg aaaaccccta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatctg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 38
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 38
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Gln Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala

210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 39
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 39
 atgaccgatac gtctgaagag caaagtagcc atcgtaaccg gcgggactct gggatcggg 60
 ttggcaatcg cagataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcggcggta ctgatgtat tgccttgtc 180
 cagcagcatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttcgcccggt ttacgaccgt cgtgaacaat gcagggatta ccgtttccag aagcgttgaa 300
 gacactacca cggaggaatg gcacaaactg ctgtccgta atctggatag tgttttttc 360
 ggcacccgtc tgggcatcca gcgcatgaag aataaaggct tggcgctag catcatcaat 420
 atgagcagta tcatggggtt cgtaggcgtat ccgacgcgg gggcatacac tcttccaag 480
 ggggcgggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgtcgttga tgaictggaa 600
 ggtgctgagg aaatgcaatc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
 aatgacgtgg catggtatcg tgtgtacctg gcactgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 40
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 40
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Ser
 85 90 95
 Arg Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Gln Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 41
 <211> 759

<212> DNA

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 41

atgaccgatac gtcgaagag caaagtagcc atcgtaacg gccggactct gggatcggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcggcggta ctgaigtat tgccttgtc 180
 cagcacgatg catcgatga agcaggctgg acgaaactgt tcgacaccac cagaggaggca 240
 ttggcccggt ttacgaccgt cgtgaacaat gcagggatta ccgttccct tagcgttgaa 300
 gacactacca cggaggaatg gcacaaactg ctgtccgta atctggatag tgttttttc 360
 ggcacccgtc tgggcattca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtggtt cgtaggcgt cgcacgctgg ggcatacac tgctccaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgctggtcga tgatctggaa 600
 ggtgctgagg aaatgatgtc acagcgtacg aaaaccccta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatctg tgtgtacctg gcactgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 42

<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 42

Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Ser
 85 90 95
 Leu Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 43

<211> 759

<212> DNA

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 43

atgaccgatac gtcgaagag caaagtagcc atcgtaacg gccggactct gggatcggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120

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gcggaatgtag gtaaaaggc cgccaaatca atcgccggta ctgatgtat tgccttgtc 180
cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
ttcgcccggt ttacgacgtt cgtgaacaat gcagggtatg cagtttcaa aagcgttgaa 300
gacactacca cggaggaatg gcacaaactg ctgtccgtta atctggatag tgttttttc 360
ggcaccctgc tgggcattca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
atgagcagta tcagtgggtt cgtaggcgat ccgacgctgg gggcatacac tgcctccaag 480
ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgtggtcga tgcctggaa 600
gggtctgagg aaatgatgtc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
aatgacgtgg catggatctg tgtgtacctg gcactgacg aatcgaaatt tgcgacgggt 720
gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 44
<211> 252
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 44
Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
1 5 10 15
Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
20 25 30
Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
35 40 45
Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
50 55 60
Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
65 70 75 80
Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
85 90 95
Lys Ser Val Glu Asp Thr Thr Thr Glu Trp His Lys Leu Leu Ser
100 105 110
Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
115 120 125
Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
130 135 140
Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
145 150 155 160
Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
165 170 175
Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
180 185 190
Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
195 200 205
Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
210 215 220
Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
225 230 235 240
Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
245 250

<210> 45
<211> 759
<212> DNA
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 45
atgaccgatc gtcgaagag caaagtagcc atcgtaaccg gcgggactct gggatcgggt 60
ttggcaatgc ccgataaatt ttagaggagg ggtgcgaaag tagttattac tggcgccac 120
gcggaatgtag gtaaaaggc cgccaaatca atcgccggta ctgatgtat tgccttgtc 180
cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
ttcgcccggt ttacgacgtt cgtgaacaat gcagggtatg cagtttcaa aagcgttgaa 300
gacactacca cggaggaatg gcacaaactg ctgtccgtta atctggatag tgttttttc 360
ggcaccctgc tgggcattca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
atgagcagta tcagtgggtt cgtaggcgat ccgacgctgg gggcatacac tgcctccaag 480
ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
gtgcgtgtca acacagtaca tccgggctat atcgaaaccc cgtggtcga tgcctggaa 600
gggtctgagg aaatgatgtc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660

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aatgacgtgg catggtatctg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
gcagaatttg tggcgacgg cggtataacc gcacagtga 759

<210> 46
<211> 252
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 46
Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
1 5 10 15
Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
20 25 30
Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
35 40 45
Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
50 55 60
Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
65 70 75 80
Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
85 90 95
Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
100 105 110
Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
115 120 125
Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
130 135 140
Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
145 150 155 160
Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
165 170 175
Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Glu
180 185 190
Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
195 200 205
Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
210 215 220
Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
225 230 235 240
Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
245 250

<210> 47
<211> 759
<212> DNA
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 47
atgaccgatc gtctgaagag caaagtagcc atcgtaaccg gcgggactct gggtatcggt 60
ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
gcggatgtag gtgaaaaggc cgccaaatca atcgccggtg ctgatgttat tgcctttgtc 180
cagcacgatg catcgatga agcaggctgg acgaaactgt tgcacaccac cgaggaggca 240
ttcgccccgg ttacgaccgt cgtgaacaat gcagggattg cagtttcaa aagcgttgaa 300
gacactacca cggagggaatg gcacaaactg ctgtccgtta atctggatag tgttttttc 360
ggcaccgcgt tgggcattca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
atgagcagta tcagtgggtt cgtaggcgtat ccgacgctgg gggcatacac tgcttccaag 480
ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
gtgcgtgtca acacagtaca tccgggctat atcgagacc ccgtgtgtga tgaatggaa 600
ggtgctgagg aaatgatgtc acagcgtacg aaaacccta tgggccactt tggcgaaccg 660
aatgacgtgg catggtatctg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
gcagaatttg tggcgacgg cggtataacc gcacagtga 759

<210> 48
<211> 252
<212> PRT
<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 48

Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Glu
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Phe Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 49

<211> 759

<212> DNA

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 49

atgaccgatac gtctgaagag caaagtagcc atcgtaacgg gcgggactct gggtatcggg 60
 ttggcaatcg cagataaatt ttagaggag ggtgcgaaag tagttattac tggtcgccac 120
 gcggaigtac gtgaaaaggc cgccaaatca atcgccggtg ctgatgttat tcgctttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cagggaggca 240
 ttggcccggt ttacgacctg cgtgaacaat gcagggattg cggttcctaa aagcgttgaa 300
 gacactacca cggagggaatg gcacaaactg ctgtccgtta atctggatag tgttttttc 360
 ggcaccgcgc tgggcattca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtgggtt ctagggcgat ccgacgctgg gggcatcac tgcctccaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgctggtcga tgatctggaa 600
 ggtgtgtagg aatgcaatc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatctg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 50

<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 50

Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala

35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Pro
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Gln Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 51
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 51
 atgaccgac gtctgaagag caaagtagcc atcgtaaccg gcgggactct gggtatcggg 60
 ttggcaatcg ccgataaatt ttagaggag ggtagcgaag tagttattac tggcgccac 120
 gcggatgtag gtgaaaaggc cgcctaatca atcgccggtg ctgattgtat tcgtttgtc 180
 cagcagatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgacgtt cgtgaacaat gcagggattg cgttttcaa aagcgttgaa 300
 gacactacca cggagggaatg gcacaaactg ctgtccgtta atctggatag tgttttttc 360
 ggcaccgcgc tgggcattca gcgcatgaag aataaaggct tggcgctag catcatcaat 420
 atgagcagta tcagtgggtt cgtaggcgat ccgacgctgg gggcatacac tgctccaag 480
 gggcggttac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgtgggtcga tgatctggaa 600
 ggtgtctgag aaatgcaatc acagcgtaac aaaacccta tgggccacat tggcgaaccg 660
 aatgacgtgg catggtctg tgtgtacctg gcatctgacg aatcgaaat tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 52
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 52
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110

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Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Gln Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 53
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 53
 atgaccgatac gtcgaagag caaagtagcc atcgtaacgc gcgggactct gggatocggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gcggatgtag gtaaaaaggc cgccaaatca atcggcggta ctgatgttat tgcctttgtc 180
 cagcacgatg cgtccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgacctg cgtgaacaat gcagggatta ccgttccaaa aagcgttgaa 300
 gacactacca cggaggaatg gcacaaactg ctgtccgita atctggatag tgttttttc 360
 ggcaccgctc tgggcattca acgcatgaag aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcatgtgggt cgtaggcgat ccgacgctgg gggcatalac tgcctccaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgttggtcga tgaatggaa 600
 ggtcgtgagg aaatgatgtc acagcgtacg aaaacccgga tgggtcactt tggcgagcca 660
 aatgatgtgg catggtatct tgtgtacctg gcattctgac aatcgaaatt tgcgacgggt 720
 gcagaatttg tggtagcgg cggtatatacc gcacagtga 759

<210> 54
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 54
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Pro
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys

180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Phe Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 55
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 55
 atgaccgatac gtcgaagag caaagtagcc atcgtaaccg gcgggactct gggatcggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaag tagttattac tggcgccac 120
 gcggatgtag gtgaaaaggc tgccaaatca atcggcggta ctgatgtgt ccgtttgtc 180
 cagcaccgat catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgaccgt cgtgaacaat gcagggattg cagttccga aagcgttgaa 300
 gacactacca cggagggaatg gcacaaactg ctgtccgita atctggatag tgttttttc 360
 ggcacccgtc tgggcattca gcgcatgaaa aataaaggct tggcgctag catcaatca 420
 atgagcagta tcagtgggtt cgtaggcgt atcgacgtgg gggcatalac tcttccaag 480
 ggggcgggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacga 540
 gtgcgtgtca acacagtaca tccgggctat atcaagacc cgtcgttga tgcctggaa 600
 ggtgctgagg aatgatgtc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatctg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 56
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 56
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Val Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Glu Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 57
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 57
 atgacgcatc gtcgaagag caaagtagcc atcgtaacgg gcgggactct gggatcggt 60
 ttggcaatcg ccgataaatl ttagaggag ggtagcgaag tagttatcac tggtagccac 120
 ggggatgtag gtgagaaggc cgcgggtca atcggcggtt ctgatgtat tgcctttgtc 180
 cagcagcatg catcgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgacgti cgtgaacaa gcagggatta ccgttccaaa aagcgttgaa 300
 gacactacca cggaggaatg gcgcaaactg atgtccgtta atctggatag tgttttttc 360
 ggcaccgcgc tgggcattca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtgggtt cgtaggcgat ccgacgctgg gggcatacac tgctccaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctgggtt gcgactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgctgggtcga tgatctggaa 600
 ggtgctgagg aatgcaatc acagcgtacg aaaaccctta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatctg tgtgtacctg gcactcgacg agtcgaaatt tgcgacgggt 720
 gcagaatttg tggtagcgg cggtataacc gcacagtga 759

<210> 58
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 58
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Arg Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Pro
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Met Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Gly Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Gln Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 59
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 59

atgaccgatc gtcgaagag caaagtagcc atcgtaacg gcgggactct gggatocgtt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaag tagttattac tggcgccac 120
 gcggatgtag gtgagaaggc cgcgggtca atcgccggtg ctgatgtat tgcctttgc 180
 cagcacgatg catccgaiga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgaccgt cgtgaacaat gcagggatta cgtttccaa aagcgttgaa 300
 gacactacca cggaggaaatg gcgcaaaactg atgtccgtta atctggatag tgttttttc 360
 ggcaccgcgtc tgggcattca gcgcatgaag aataaaggct tggcgctag catcatcaat 420
 atgagcagta tcagtgggtt cgtaggcgat ccgacgctgg gggcatacac tgcctccaag 480
 gggcggtac gtatcatgtc gaaaagcgca gcgcgggtt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgtcgggtcga tgatcggaa 600
 ggtgctgagg aaatgatgtc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatctg tgtgtacctg gcactgacg agtcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 60

<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 60

Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Arg Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gin His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Met Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gin Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Gly Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gin
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gin
 245 250

<210> 61

<211> 759

<212> DNA

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 61

atgaccgatc gtcgaagag caaagtagcc atcgtaacg gcgggactct gggatocgtt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaag tagttattac tggcgccac 120
 gcggatgtag gtgagaaggc cgcgggtca atcgccggtg ctgatgtat tgcctttgc 180
 cagcacgatg catccgaiga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgaccgt cgtgaacaat gcagggatta cgtttccaa aagcgttgaa 300
 gacactacca cggaggaaatg gcgcaaaactg atgtccgtta atctggatag tgttttttc 360
 ggcaccgcgtc tgggcattca gcgcatgaag aataaaggct tggcgctag catcatcaat 420

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atgagcagta tcagtgggtt cgtaggcga cgcagcgtgg gggcatacac tgctccaag 480
ggggcggtac gtatcatgtc gaaaagcgca gcgctgggtt gcgcactgaa ggactacga 540
gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgtggtcga tgatctggaa 600
ggtgctgagg aatgcaatc acagcgtacg aaaaccccta tgggccacat tggcgaaccg 660
aatgacgtgg catggatctg tgtgtacctg gcatctgacg agtcgaaatt tgcgacgggt 720
gcagaatttg tggcgcacgg cgggtatacc gcacagtga 759

<210> 62
<211> 252
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 62
Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
1 5 10 15
Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
20 25 30
Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
35 40 45
Arg Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
50 55 60
Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
65 70 75 80
Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Ser
85 90 95
Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Met Ser
100 105 110
Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
115 120 125
Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
130 135 140
Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
145 150 155 160
Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Gly Cys Ala Leu
165 170 175
Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
180 185 190
Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Gln Ser Gln
195 200 205
Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
210 215 220
Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
225 230 235 240
Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
245 250

<210> 63
<211> 759
<212> DNA
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 63
atgaccgatac gtcgaagag caaagtagcc atcgtaacgg gcgggactct gggatatcgt 60
ttggcaatcg ccgataaatt ttagaggag ggtcgaaag tagttattac tggcgcac 120
gcggatgtag gtgagaaggc cgcgcgttca atcggcgcta ctgatgttat tgcctttgtc 180
cagcagcatg catccgatga agcaggctgg acgaaacigt tcgacaccac caggaggca 240
ttcggcccg ttagaccgt cgtgaacaat gcagggattg cagttccaaa aagcgttgaa 300
gacactacca cggaggaaatg gcgcaaaactg atgtccgta atctggatag tgtttttc 360
ggcaccgctc tgggcattca gcgcatgaag aataaaggct tggcgctag catcatcaat 420
atgagcagta tcagtgggtt cgtaggcga cgcagcgtgg gggcatacac tgctccaag 480
ggggcggtac gtatcatgtc gaaaagcgca gcgctgggtt gcgcactgaa ggactacga 540
gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgtggtcga tgatctggaa 600
ggtgctgagg aatgcaatc acagcgtacg aaaaccccta tgggccacat tggcgaaccg 660
aatgacgtgg catggatctg tgtgtacctg gcatctgacg agtcgaaatt tgcgacgggt 720
gcagaatttg tggcgcacgg cgggtatacc gcacagtga 759

<210> 64

<211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 64
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Arg Ser Ile Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Pro
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Met Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Gly Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 65
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 65
 atgaccgatac gtctgaagag caaagtagcc atcgtaaccg gcgggactct gggatcgggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaag tagttattac tggcgccac 120
 gcggatgtag gtgagaaggc cgcccggtca atcggcggta ctgatgttat tcgctttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgaccgt cgtgaacaat gcagggatg cagttccaaa aagcgttgaa 300
 gacactacca cggaggaatg gcgcaaaactg atgtccgtta atctggatag tgttttttc 360
 ggcacccgctc tgggcattca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtggtt cgtaggcgat ccgacgctgg gggcatacac tgcttccaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctgggtt ggcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgctggtcga tgatctggaa 600
 ggtgtctagg aaatgcaatc acagcgtaacg aaaacccta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatctg tgtgtacctg gcatctgacg agtcgaaatt tgcgacgggt 720
 gcagaatttg tggcgcacgg cgggtatacc gcacagtga 759

<210> 66
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 66
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr

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1      5      10     15
Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
      20     25     30
Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
      35     40     45
Arg Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
      50     55     60
Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
65     70     75     80
Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Pro
      85     90     95
Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Met Ser
      100    105    110
Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
      115    120    125
Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
      130    135    140
Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
145    150    155    160
Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Gly Cys Ala Leu
      165    170    175
Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
      180    185    190
Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Gln Ser Gln
      195    200    205
Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
      210    215    220
Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
225    230    235    240
Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
      245    250

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<210> 67
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

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<400> 67
atgaccgac gtctgaagag caaagtagcc atcgtaacg gcgggactct gggatcggt 60
ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
gcggaatgag gtgagaaggc cgcccggtca atcgccggtg ctgatgtat tgccttgtc 180
cagcacgatg catcogatga agcaggctgg acgaaactgt tgcacaccac cgaggaggca 240
ttcgcccggt ttacgacgt cgtgaacaat gcagggattg cagtttcaa aagcgttgaa 300
gacactacca cggaggaatg gcgcaaaactg atgtccgtta atctggatag tgttttttc 360
ggcaccgctc tgggcattca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
atgagcagta tcagtgggtt cgtaggcgat ccgacgcgg gggcatacac tgcttccaag 480
ggggcggtac gtatcagtgc gaaaagcgca gcgtgggtt gcgcactgaa ggactacgat 540
gtgcgltgca acacagtaca tccgggctat atcaagaccc cgtgggtcga tgatctggaa 600
gggtctgagg aaatgatgtc acagcgtagc aaaaccccta tgggccacat tggcgaaccg 660
aatgacgtgg catggaatcg tgtgtacctg gcatctgacg agtcgaaatt tgcgacgggt 720
gcagaatttg tggcgacgg cggtataacc gcacagtga 759

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<210> 68
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

```

<400> 68
Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
1      5      10     15
Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
      20     25     30
Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
      35     40     45
Arg Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
      50     55     60
Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
65     70     75     80

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Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Met Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Gly Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 69
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 69
 atgaccgatac gtctgaagag caaagtagcc atcgtaaccg gcgggactct gggatatcgg 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaag tagttattac tggcgccac 120
 gcggatgtag gtgagaaggc cgcccggtca atcgccggtg ctgatgttat tgcgtttg 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttccgcccgg ttacgaccgt cgtgaacaat gcagggattg cagtttccaa aagcgttgaa 300
 gacactacca cggaggaatg gcgcaaaactg atgtccgtta atctggatag tgtttttc 360
 ggcaccgctc tgggcattca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcatgggtt cgtaggcgat ccgacgctgg gggcatacac tgcctccaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctgggtt gcgcactgaa ggactacgat 540
 gtgcgtgta acacagtaca tccgggctat atcaagaccc cgttggtcga tgatcggaa 600
 ggtgctgagg aaatgcaatc acagcgtagc aaaaccctta tgggccacat tggcgaaccg 660
 aatgacgtgg catgcatctg tgtgtacctg gcatctgacg agtcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 70
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 70
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Arg Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Met Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys

145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Gly Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Gln Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Tyr Thr Ala Gln
 245 250

<210> 71
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 71
 atgaccgatac gtctgaagag caaagtagcc atcgtaaccg gcgggggact gggtatcgg 60
 ttggcaatcg cagataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcgcggtta ctgatgtat tgccttgc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttcgcccggt ttacgaccgt cgtgaacaat gcagggatta ccgttccaaa aagcgttgaa 300
 gacactacca cggaggaatg gcacaaactg ctgtccgta atctggatag tgtttttc 360
 ggcacccgtc tgggcatcca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtgggtt cgtaggcgtat ccgacgctgg gggcatacac tgctccaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgt 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgctggtcga tgaictggaa 600
 ggtgctgagg aaatgcaatc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatctg tgtgtacctg gcactgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 72
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 72
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Gly
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Pro
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Gln Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220

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Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
225 230 235 240
Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
245 250

<210> 73
<211> 759
<212> DNA
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 73
atgaccgatc gtctgaagag caaagtagcc atcgtaacgg gcgggggact gggtatcggg 60
ttggcaatcg ccgataaatt ttagaggag gggtcgaaag tagttattac tggcgccac 120
gaggatgtag gtgaaaaggc cgccaaatca atcggcggtg ctgatgttat tggcttggc 180
cagcacgatg catcggatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
ttcgcccggt ttacgacgtt cgtgaacaat gcagggttg cagtttcaa aagcgttga 300
gacactacca cggagggaatg gcacaaactg ctgtccgtta atctggatag tgttttttc 360
ggcaccgttc tgggcatca ggcgatgaag aataaagggt tgggcgctag catcatcaat 420
atgagcagta tcagtgggtt cgtaggcgat ccgacgtgg gggcatcac tgcctccaag 480
ggggcggtac gtatcatgtc gaaaagcgca gcgtggatt gcgcactgaa ggactacgat 540
gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgtggtcga tgaatcggaa 600
gggctgagg aatgatgtc acagcgtacg aaaaccccta tggccacat tggcgaaccg 660
aatgacgtgg catggtctg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 74
<211> 252
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 74
Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Gly
1 5 10 15
Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
20 25 30
Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
35 40 45
Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
50 55 60
Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
65 70 75 80
Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
85 90 95
Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
100 105 110
Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
115 120 125
Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
130 135 140
Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
145 150 155 160
Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
165 170 175
Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
180 185 190
Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
195 200 205
Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
210 215 220
Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
225 230 235 240
Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
245 250

<210> 75
<211> 759
<212> DNA

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 75

atgaccgac gtctgaagag caaagtagcc atcgtaaccg gcgggggact gggtatcggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggtcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcgcggtga ctgatgttat tgcctttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgacctg cgtgaacaat gcagggattg cggttcctaa aagcgttgaa 300
 gacactacca cggaggaaatg gcacaaactg ctgtccgtta atctggatag tgttttttc 360
 ggcacccgtc tgggcattca gcgcatgaag aataaagggt tgggcgctag catcatcaal 420
 atgagcagta tcagtgggtt cgtaggcgtat ccgacgctgg gggcatalac tgctccaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgtggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgtgtgtcga tgatctggaa 600
 ggtgctgagg aatgtcaatc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatctg tgtgtacctg gcattcgacg aatcgaaatt tgcgaagggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 76

<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 76

Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Gly
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Pro
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Gln Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 77

<211> 759

<212> DNA

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 77

atgaccgac gtctgaagag caaagtagcc atcgtaaccg gcgggggact gggtatcggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggtcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcgcggtga ctgatgttat tgcctttgtc 180

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cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgacctg cgtgaacaat gcagggattg tggttcciaa aagcgttgaa 300
 gacactacca cggagggaatg gcacaaactg ctgtccgtta atctggatag tgttttttc 360
 ggcacccgtc tgggcattca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtggtt cgtaggcgtat ccgacgtgg gggcatacac tgcctccaag 480
 ggggcggtaac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagacct cgctggctga tgatctggaa 600
 ggtgctgagg aaatgcaatc acagcgtacg aaaaccctta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatctg tgtgtacctg gcactgacg aatcgaaatt tgcgaacgggt 720
 gcagaatttg tggtagcgg cgggtatacc gcacagtga 759

<210> 78
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 78
 Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Gly
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Val Val Pro
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Gln Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 79
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 79
 atgaccgatc gtctgaagag caaagtagcc atcgtaaccg gcggggctct gggatcgggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaag tagttattac tggtcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcgccggtg ctgatgttat tgcctttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgacctg cgtgaacaat gcagggatta ccgttccaaa aagcgttgaa 300
 gacactacca cggagggaatg gcacaaactg ctgtccgtta atctggatag tgttttttc 360
 ggcacccgtc tgggcattca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtggtt cgtaggcgtat ccgacgtgg gggcatacac tgcctccaag 480
 ggggcggtaac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagacct cgctggctga tgatctggaa 600
 ggtgctgagg aaatgcaatc acagcgtacg aaaaccctta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatctg tgtgtacctg gcactgacg aatcgaaatt tgcgaacgggt 720

gcagaatttg tggtcgacgg cgggtatacc gcacagtga 376247016
759

<210> 80
<211> 252
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 80
Met Thr Asp Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Val
1 5 10 15
Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
20 25 30
Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
35 40 45
Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
50 55 60
Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
65 70 75 80
Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Pro
85 90 95
Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
100 105 110
Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
115 120 125
Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
130 135 140
Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
145 150 155 160
Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
165 170 175
Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
180 185 190
Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Gln Ser Gln
195 200 205
Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
210 215 220
Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
225 230 235 240
Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
245 250

<210> 81
<211> 759
<212> DNA
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 81
atgaccgacg gtcgaagca caaagtagcc atcgtaacgg gcgggacict gggtatcggt 60
ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggtcgccac 120
gcggatgtag gtgaaaaggc cgccaaatca atcggcggta ctgatgtat tgccttfgtc 180
cagcacgatg catccgatga agcaggcigg acgaaactgt tgcacaccac cgaggaggca 240
ttcggcccggt ttacgaccgt cgtgaacaat gcagggattg cagtticcaa aagcgttgaa 300
gacactacca cggaggaatg gcacaaactg ctgtccgita atctggatag tgitttttc 360
ggcaccgcgt tgggcattca gcgcatgaag aataaaggct tgggcgctag catcatcaat 420
atgagcagta tcagtgggtt cgtaggcgat cgcacgctgg gggcatacac tgctccaag 480
ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt ggcactgaa ggactacgat 540
gtcgtgtca acacagtaca tccgggctat atcaagacc cgtgggtcga tgatctggaa 600
gggtctgagg aaatgatgtc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
aatgacgtgg catggatctg tgtgtacctg gcatctgacg aatcgaaaat tgcgacgggt 720
gcagaatttg tggtcgacgg cgggtatacc gcacagtga 759

<210> 82
<211> 252
<212> PRT
<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 82

Met Thr Asp Arg Leu Lys His Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 83

<211> 759

<212> DNA

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 83

atgaccgatc gtctgaaggg caaagtagcc accgtaaccg gcgggactct gggtatcggt 60
 ttggcaatcg cggataaatt ttagaggag ggtagcgaag tagttattac tggtagtcac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcgccggca ctgatgtat tgcgtttgtc 180
 cagcatgatg catcgaatga agcaggctgg acgaaactgt tcgacaccac caggaggcca 240
 ttggcccggt ttacgactgt cgtgaacaat gcagggatta cagttccaa aagcgttgaa 300
 gacactacca cggaggaatg ggcgaaactg ctgtccgtta atctggatgg tgttttttc 360
 ggcaccgctc tgggcattca gcgcatgaaa aataaaggct tggcgctag catcatcaat 420
 atgagcagta ttgagggtgt ctagggcgat ccgacgctgg gggcatacag cgctccaag 480
 gggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgttggtoga tgaatggaa 600
 ggtgctgagg aaatgatgtc acagcgtagc aaaacccta tgggccacat tgggaaccg 660
 aatgacatcg catggatctg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg ttgtgacg cggtatacc gcacagtga 759

<210> 84

<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 84

Met Thr Asp Arg Leu Lys Gly Lys Val Ala Thr Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45

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Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Glu Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Ser Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 85
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 85
 atgacgagtc gtctgaaggc caaagtagcc atcgtaaccg gcgggactct gggatcggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtcgaaag tagttattac tggcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcgcgcgca ctgatgttat tgcgtttgtc 180
 cagcagcatg catccgaiga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttcgcccggt ttacgaccgt cgtgaacaat gcagggattg cagtttcaa aagcgttgaa 300
 gacactacca cggaggaatg gcacaaactg ctgtccgtta atctggatag tttttttc 360
 ggcaccggtc tgggcattca gcgcataaaa aataaaggct tggcgctag catcatcaat 420
 atgagcagta tcagcgggtt cgtaggcgat ccgacgctgg gggcatacac tgctccaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atccgcacc cgtggtoga tgaictggaa 600
 ggtgctgagg aaatgatgtc acagcgtagc aaaaacccta tgggccacat tggcgaaacc 660
 aatgacgtgg catggtatgt tgtgtacctg gcactgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 86
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 86
 Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg

115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Arg
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 87
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 87
 atgacogatc gtctgaaggg caaagtagcc atcgtaaccg gcgggactct gggatccggt 60
 ttggcaatcg cggataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gggatgtag gtgaaaaggc cgcaaatca atcgcgcgca ctgatgtat tgcgtttgc 180
 cagcacgatg catccgaaga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgacgtt cgtgaacaa gcagggtatg cagtttcaa aagcgttgaa 300
 gacactacca cggagggaatg gcgtaaactg ctgtccgtta atctggatag tgttttttc 360
 ggcaccgcgc tgggcatlca gcgcatgaaa aataaaggct tgggcgctag catcatcaac 420
 atgagcagta tcagtgggtt cgtaggcgat ccgacgctgg gggcatacac cgcticcaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctggat gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgctggtcga tgatctggaa 600
 ggtgcgtagg aaatgatgtc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
 aatgacatcg catggatctg tgtgtacctg gcactgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 88
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 88
 Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190

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Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 89
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 89
 atgaccgac gtctgaaggg caaagtagcc atcgtaacgg gcgggactct gggtatcggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcgcgggca ctgatgtat tgcctttgc 180
 cagcacgatg catccgaiga agcaggctgg acgaaactgt tgcacaccac cgaggaggca 240
 ttccggccgg ttacgacgti cgtgaacaat gcagggttg cagtttcaa aagcgttgaa 300
 gacactacca cggaggaatg gcgtaaactg ctgtccgtta atctggatag tgttttttc 360
 ggcaccgcgc tgggcatlca gcgcatgaaa aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtgggtt cgtaggcgat cgcacgctgg gggcatacaa cgctccaag 480
 ggggcggtac gtaicagtgc gaaaagcgca gcgtggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgctggctga tgaictggaa 600
 ggtgctgagg aaatgatgtc acagcgtacg aaaaccccta tgggccacat tggcgaaccg 660
 aatgacatcg catggatcgt tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggtcgacgg cgggtatacc gcacagtga 759

<210> 90
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 90
 Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Asn Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 91
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 91
 atgaccgac gtcgaagg caaagtagc atcgtaacg gcgggactc gggatcggt 60
 ttggcaatc cgataaat ttagaggag ggtgcgaag tagttattc tggcgccac 120
 gcggaigtg gtagaaagg cgcacaaatc atcgccggc ctgatgtat tgccttgc 180
 cagcagatg catccgatg agcaggctg acgaaactg tcgacaccac caggaggca 240
 ttggcccg ttacaccgt cgtgaacaat gcagggattg cagtticac aagcgtgaa 300
 gacactacca cggaggaatg gcgtaaactg ctgtccgtt atctggatg tgtttttc 360
 ggcaccgctc tgggcattc gcgcatgaaa aataaaggct tggcgctag catcatcaat 420
 atgagcagta tcagtgggt cgtagggat cgcagctgg ggcatacac tcttccaag 480
 ggggcgtac gtatcatgc gaaaagcga gcgctggat gcgcactga ggactacat 540
 gtgcgtgta acacagtaca tccgggtat atccgaccc cgtggtoga tgatcggaa 600
 ggtgctgagg aatgatgtc acagcgtacg aaaacccta tggccacat tggcgaaccg 660
 aatgacatc catggatcg tgtgtaccg gcactgacg aatcgaatt tgcgacgggt 720
 gcagaattg tggcgacgg cgggtatacc gcacagta 759

<210> 92
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 92
 Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Ser Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Arg
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 93
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 93

atgaccgac gtctgaaggg caaagtagcc atcgtaaccg gcgggactct gggatcgggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gcggatgtag gtagaaaggc cgcgaatca atcgcgcgca ctgatgttat tgcctttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttccgcccgg ttaagaccgt cgtgaacaat gcagggattg cagtttccaa aagcgttgaa 300
 gacactacca cggaggaatg gcgtaaactg ctgtccgtta atctggatag tgttttttc 360
 ggcaccgcgtc tgggcattca gcgcatgaaa aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtgggtt cgtaggcgat ccgatgcctg gggcatacac tgcctccaag 480
 gggcgctac gtatcatgtc gaaaagcgca gcgcgtgati gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgtcgttoga tgccttgaa 600
 ggtgctgagg aaatgatgtc acagcgtaac aaaaccoccta tgggccacat tggcgaaacc 660
 aatgacatcg catggatcgt tctgtaccgt gcactcgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 94

<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 94

Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Met Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 95

<211> 759

<212> DNA

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 95

atgaccgac gtctgaaggg caaagtagcc atcgtaaccg gcgggactct gggatcgggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gcggatgtag gtagaaaggc cgcgaatca atcgcgcgca ctgatgttat tgcctttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttccgcccgg ttaagaccgt cgtgaacaat gcagggattg cagtttccaa aagcgttgaa 300
 gacactacca cggaggaatg gcgtaaactg ctgtccgtta atctggatg tgttttttc 360
 ggcaccgcgtc tgggcattca gcgcatgaaa aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtgggtt cgtaggcgat ccgacgcctg gggcatacaa cgtctccaag 480

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ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgctggtcga tgatctggaa 600
gggtctgagg aaatgatgtc acagcgtagc aaaacccta cgggccacat tggcgaaccg 660
aatgacatcg catggatctg tigtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
gcagaatttg tggcgaagg cgggtatacc gcacagtga 759

<210> 96
<211> 252
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 96
Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
1 5 10 15
Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
20 25 30
Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
35 40 45
Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
50 55 60
Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
65 70 75 80
Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
85 90 95
Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
100 105 110
Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
115 120 125
Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
130 135 140
Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Asn Ala Ser Lys
145 150 155 160
Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
165 170 175
Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
180 185 190
Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
195 200 205
Arg Thr Lys Thr Pro Thr Gly His Ile Gly Glu Pro Asn Asp Ile Ala
210 215 220
Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
225 230 235 240
Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
245 250

<210> 97
<211> 759
<212> DNA
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 97
atgaccgata gtctgaaggg caaagtagcc atcgtaacgg gcgggactct gggtatcggt 60
ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggtcgccac 120
gcggatgtag gtgaaaaggc cgccaaatca atcggcgcca ctgatgtat tgcctttgtc 180
cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
ttcggcccggt ttacgacgti cgtgaacaa gcagggattg cagtttcaa aagcgttgaa 300
gacactacca cggaggaatg gcgtaaactg ctgtccgtta atctggatgg tgttttttc 360
ggcaccggc tgggcatcca gcgcatgaaa aataaaggct tgggcgctag catcatcaat 420
atgagcagta tcagtgggtt cgtaggcgat ccgacgctgg gggcatacaa cgcttccaag 480
ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgctggtcga tgatctggaa 600
gggtctgagg aaatgatgtc acagcgtagc aaaacccta tgggccacat tggcgaaccg 660
aatgacatcg catggatctg tigtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
gcagaatttg tggcgaagg cgggtatacc gcacagtga 759

<210> 98
<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 98

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Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
1      5      10     15
Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
20     25     30
Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
35     40     45
Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
50     55     60
Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
65     70     75     80
Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
85     90     95
Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
100    105    110
Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
115    120    125
Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
130    135    140
Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Asn Ala Ser Lys
145    150    155    160
Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
165    170    175
Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
180    185    190
Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
195    200    205
Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
210    215    220
Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
225    230    235    240
Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
245    250

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<210> 99

<211> 759

<212> DNA

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 99

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ttggcaatcg ccgataaaatt ttagaggag ggtgcgaaag tagttattac tggtcgccac 120
gaggatgtag gtgaaaaggc cgccaaatca atcggcggca ctgatgttat tgcgtttgtc 180
cagcacgatg catcgaatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
ttcggccggg ttacgacctg cgtgaacaa gcagggattg cagtttccaa aagcgttgaa 300
gacactacca cggaggaatg gcgtaaactg ctgtccgita atctggatgg tgttttttc 360
ggcaccgcgc tgggcattca gcgcatgaaa aataaaggct tgggcgctag catcatcaat 420
atgagcagta tcagtgggtt cgtaggcgat ccgacgctgg gggcatacaa cgtctccaag 480
ggggcggtag gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
gtgcgtgta acacagtaca tccgggctat atcaagaccc cgtcgttga tgatctggaa 600
ggtgctgagg aaatgatgtc acagcgtacg aaaacccta tgggccactt tggcgaaccg 660
aatgacatcg cgtggatctg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
gcagaatttg tggtcgacgg cgggtatacc gcacagtga 759

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<210> 100

<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 100

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Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
1      5      10     15

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Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Asn Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Phe Gly Glu Pro Asn Asp Ile Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 101
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 101
 atgaccgcatc gtctgaaggc caaagtagcc atcgtaacgc gcgggactct gggtatcggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagtattac tggcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcgccggca ctgatgtat tgcgttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttccgcccgg ttacgacctg cgtgaacaat gcagggatg cagtttccaa aagcgttgaa 300
 gacactacca cggaggaatg gcgtaaactg ctgtccgtta atctggatgg tgttttttc 360
 ggcacccgtc tgggcattca gcgcatgaaa aataaaggct tggcgctag catcatcaat 420
 atgagcagta tcatgtgggt cgtaggcgat ccgacgctgg gggcatacac cgttccaag 480
 gggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgtcgttcga tgatctggaa 600
 ggtcgtgagg aaatgatgtc acagcgtacg aaaaccctta tgggccacat tggcgaaccg 660
 aatgacatcg catggatctg tgtgtacctg gcactcgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cggtataacc gcacagtga 759

<210> 102
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 102
 Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser

85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 103
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 103
 atgacagatc gtcgaaggg caaagtagcc atcgaaccg gcgggactct gggatcggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcgccggca ctgaigtat tgcgtttgc 180
 cagcacgalt catccgatga agcaggctgg acgagactgt tgcacaccac cgaggaggca 240
 ttccgcccgg ttacgacgti cgtgaacaat gcagggtatt cagtttccaa aagcgttgaa 300
 gacactacca cggaggaatg gcgtaaactg ctgtccgtta atctggatag tgttttttc 360
 ggcaccogtc tgggcattca gcgcatgaaa aataaaggct tggcgctag catcatcaat 420
 atgagcagta tcaatgggtt cgtaggcgat ccgacgctgg gggcatacac cgctccaag 480
 gggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagacc cgctggtcga tgaatggaa 600
 ggtgctgagg aaatgatgtc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
 aatgacgtgg catggatcgt tgtgtacctg gcactgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 104
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 104
 Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Arg Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160

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Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 105
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 105
 atgaccgatc gtcgaaggg caaagtagcc atcgaaccg gcgggactct gggtatcggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggtcgccac 120
 gcggatgtag gtgaaaggc cgcagatca atcgcgggca ctgatgtat tcgcttgtc 180
 cagcacgatc catcgaatg agcaggctgg acgaaactgt ttgacaccac caggaggca 240
 ttggcccggt ttacgacctg cgtgaacaat gcagggattg cagtttcaa aagcgttgaa 300
 gacactacca cggaggaatg gcacaaactg ctgtccgtta atctggatag tgtttttc 360
 ggcacccgtc tggcattca gcgcatgaaa aataaaggct tggcgctag catcatcaat 420
 atgagcagta tcagtgggtt cgtaggcgt cgcacgctgg gggcatacac tgcttccaag 480
 gggcggtac gtaicgatgc gaaaagcga gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgta acacagtaca tccgggctat atcaagaccc cgtgtgtcga tgactggaa 600
 ggtgctgagg aatgatgtc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
 aatgaactgg catggtatcgt tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 106
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 106
 Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Arg Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly

225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 107
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 107
 atgaccgatac gtcgaaggg caaagtagcc atcgtaaccg gcgggactct gggatatcgtt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gcggatgtag gtgaaaaggc cgcgaaatca atcggcggca ctgatgtat tgcctttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttgcggccgg ttacgaccgt cgtgaacaat gcaggggattg cagtttccaa aagcgttgaa 300
 gacactacca acgaggaatg gcgtaaactg ctgtccgita atctggatgg tgttttttc 360
 ggcaccgcgc tgggcattca gcgcataaaa aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtgggtt cgtaggcgat ccgacgctgg gggcatacaa cgcttccaag 480
 ggggcggtac gtaicattgc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagacct cgctggtcga tgatctggaa 600
 ggtgcigagg aatatgatgc acagcgtacg aaaacccctg tgggccacat tggcgaccg 660
 aatgacatcg catgcatctg tgtgtacctg gcactgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggtcgacgg cgggtatacc gcacagtga 759

<210> 108
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 108
 Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Asn Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Val Gly His Ile Gly Glu Pro Asn Asp Ile Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 109
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 109

atgaccgac gtctgaaggg caaagtagcc atcgtaaccg gcgggactct gggatcggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gcggatgtag gtggcaaggc cgccaaatca atggcggca ctgatgttat tcgcttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgaccgt cgtgaacaat gcagggattg cagtttccaa aagcgttgaa 300
 gacactacca cggagggaatg gcacaaactg ctgtccgita atctggatag tgttttttc 360
 ggcaccgcgc tgggcattca gcgcatgaaa aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtgggtt cgtaggcgat ccgacgttgg gggcatacac tgcctccaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgctggtcga tgaatcgaa 600
 ggtgctgagg aaatgatgtc acagcgtacg aaaaccoccta tgggcccacat cggcgaaccg 660
 aatgacgtgg catggatctg tgtgtacctg gcactgacg aatcgaaat tgcgacgggt 720
 gcggaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 110

<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 110

Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Gly Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 111

<211> 759

<212> DNA

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 111

atgaccgac gtctgaaggg caaagtagcc atcgtaaccg gcgggactct gggatcggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggcgccac 120
 gcggatgtag gtggcaaggc cgccaaatca atggcggca ctgatgttat tcgcttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240

376247016

ttcggcccg ttacgaccgt cgtgaacaat gcagggatig cggtttccaa aagcgttgaa 300
gacactacca cggaggaatg gcacaaactg ctgtccgta atctggatag tgttttttc 360
ggcaccgcgc tgggcattca ggcgatgaaa aataaaggct tgggcgctag catcatcaat 420
atgagcagta tcagtgggtt cgtaggcgtat ccgacgcgtg gggcatacac tgcctccaag 480
ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
gtgcgtgtca acacagtaca tccgggctat atccgtaccc cgttggtcga tggctcggaa 600
gggtcgtagg aaatgatgtc acagcgtacg aaaaccctta tgggccacat tggcgaaccg 660
aatgacgtgg catggatctg tgtgtacctg gcactgacg aatcgaaatt tgcgacgggt 720
gcagagtttg tggtcgacgg cgggtatacc gcacagtga 759

<210> 112
<211> 252
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 112
Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
1 5 10 15
Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
20 25 30
Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Gly Lys Ala Ala
35 40 45
Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
50 55 60
Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
65 70 75 80
Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
85 90 95
Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
100 105 110
Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
115 120 125
Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
130 135 140
Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
145 150 155 160
Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
165 170 175
Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Arg
180 185 190
Thr Pro Leu Val Asp Gly Leu Glu Gly Ala Glu Glu Met Met Ser Gln
195 200 205
Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
210 215 220
Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
225 230 235 240
Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
245 250

<210> 113
<211> 759
<212> DNA
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 113
atgaccgatc gtctgaaggg caaagtagcc atcgtaacgg gcgggactct gggatcgggt 60
ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggctgtcac 120
gcggatgtag gtgaaaaggc cgccaaatca atcggcggca ctgatgtat tgcgtttgc 180
cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
ttcggcccg ttacgaccgt cgtgaacaat gcagggatig cggtttccaa aagcgttgaa 300
gacactacca cggaggaatg gcgtaaactg ctgtccgta atctggatg tgttttttc 360
ggcaccgcgc tgggcattca ggcgatgaaa aataaaggct tgggcgctag catcatcaat 420
atgagcagta ttgaggggtt cgtaggcgtat ccgacgcgtg gggcatacag cgttccaag 480
ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgttggtcga tgcctcggaa 600
gggtcgtagg aaatgatgtc acagcgtacg aaaaccctta tgggccacat tggcgaaccg 660
aatgacatcg catggatctg tgcgtacctg gcactgacg aatcgaaatt tgcgacgggt 720
gcagaatttg tggtcgacgg cgggtatacc gcacagtga 759

<210> 114
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 114
 Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Glu Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Ser Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
 210 215 220
 Trp Ile Cys Ala Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 115
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 115
 atgaccgatac gtctgaaggg caaagtagcc atcgtaaccg gcgggactct gggtatcgg 60
 ttggcaatcg cagataaatt ttagaggag ggtgcgaaag tagttattac tggctgcac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcgcgga ctagtattat tgccttctc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac agaggaggca 240
 ttccggccgg ttacgacctg cgtgaacaat gcagggattg cagtttccaa aagcgttgaa 300
 gacactacca cggaggaatg gogtaaacig ctgtccgta atctggatgg tgttttttc 360
 ggcaccgcgc tgggcattca gcgcataaaa aataaaggct tggcgctag catcatcaat 420
 atgagcagta ttgatgggtt cgtaggcgt cgcagcctgg gggcatacaa cgctccaag 480
 gggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgtcgttoga tgatctggaa 600
 ggtgctgagg aaatgatgtc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
 aatgacatcg catggaatcg tgtgtacctg gcatctgacg aatogaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cggtatacc gcacagtga 759

<210> 116
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 116

Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Asp Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Asn Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 117

<211> 759

<212> DNA

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 117

atgaccgatac gtctgaaggc caaagtagcc atcgtaaccg gcgggactct gggtatcggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtcgaaag tagttattac tggctgtcac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcgcgcgca ctgatgttat tgcgtttgtc 180
 cagcacgatg catcgatga agcaggctgg acgaaactgt tcgacaccac cagaggaggca 240
 ttcgcccggt ttacgaccgt cgtgaacaat gcagggatta cagtttccaa aagcgttgaa 300
 gacactacca cggaggaatg gcgtaaactg ctgtccgtta atctggatgg tgttttttc 360
 ggcacccgtc tgggcgttca gcgcatgaaa aataaaggct tgggcgctag catcatcaat 420
 atgagcagta ttgaggggtt cgtaggcgat ccgacgctgg gggcatacag cgcttccaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcggtga acacagtaca tcggggctat atcaagaccc cgttggtcga tgatctgaa 600
 ggtgctgagg aaatgatgtc acagcgtacg aaaaccctta tgggccacat tggtaaccg 660
 aatgacatcg catggatctg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 118

<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 118

Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala

50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Val Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Glu Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Ser Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 119
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 119
 atgaccgacg gtcgaaggg caaagtagcc atcgtaaccg gcgggactct gggtatcggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggctgtcac 120
 gcggtatgag gtgaaaaggc cgccaaatca atcgcgcgca ctgatgttat tgcgtttgic 180
 cagcatgatg catcgatga agcaggctgg acgaaactgt tgcacaccac cgaggaggca 240
 ttgcggccgg ttacgactgt cgtgaacaat gcagggatg cagtttccaa aagcgttgaa 300
 gacactacca cggagggaatg gcgtaaactg ctgtccgita atctggatgg tgttttttc 360
 ggcaccgcgc tgggcattca gcgcatgaaa aataaaggct tgggcgctag catcatcaat 420
 atgagcagta ttgagggggt cgtaggcgat cgcacgctgg ggcatacag cgtttccaag 480
 ggggcggtag gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgtctgtcga tgcctggaa 600
 ggtgctgagg aaatgatgtc acagcgtacg aaaacccta tgggccacat tgggaaccg 660
 aatgacatcg catggatcgt tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggtcgacgg cgggtatacc gcacagtga 759

<210> 120
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 120
 Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125

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Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Glu Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Ser Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 121
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 121
 atgaccgatac gtctgaaggg caaagtagcc atcgtaaccc gcgggactct gggatatcgtt 60
 ttggcaatcg cgcataaatt ttagaggag ggtgcgaaag tagttattac tggctgcac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcgccgtca ctgatgtat tgccttctc 180
 cagcacgagc catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgaccgt cgtgaacaat gcagggatg cagtttccaa aagcgttgaa 300
 gacactacca cggaggaatg gcgtaaactg ctgtccgtta atctggatgg tgttttttc 360
 ggcaccctgc tgggcattca gcgcataaaa aataaaggct tgggcgctag catcatcaat 420
 atgagcagta ttgagggtt cgtaggcgt cgcacgctgg gggcatacag cgttccaag 480
 ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagaccc cgtcgttoga tgcctcggaa 600
 ggtgctgagg aaatgatgtc acagcgtacg aaaaccoccta tgggccacat tggcgaaccg 660
 aatgacatcg catgcatcgt tctgtacctg gcactcgaag aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgcagg cgggtatacc gcacagtga 759

<210> 122
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 122
 Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Val Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Glu Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Ser Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln

195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 123
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 123
 atgaccgatac gtctgaaggc caaagtagcc atcgtaaccg gcgggactct gggtatcggg 60
 ttggcaatcg ccgataaatt ttagaggag ggtcgaaaag tagttattac tggctgtcac 120
 gtggatgtag tggaaaaggc cccagatca atcggcggca ctgatgttat tcgcttgtc 180
 cagcagcatg catccgatga agcaggctgg acgaaactgt tcgacaccac caggaggca 240
 ttggcccggt ttacgaccgt cgtgaacaat gcagggattg cagtttccaa aagcgttgaa 300
 gacactacca cggaggaatg gcgtaaactg ctgtccgita aictggatgg tgttttttc 360
 ggcaccgcgtc tgggcattcg gcgcatgaaa aataaaggct tgggcgctag catcatcaat 420
 atgagcagta ttgaggggct ctaggcgat cgcagcctgg ggcatacaa cgcttcaaag 480
 ggggcggtac gcatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgtgca acacagtaca tccgggctat atcaagaccc cgcgtgtcga tgatctgaaa 600
 ggtgctgagg aatgatgtc acagcgtacg aaaacccta tgggccactt tggcgaaccg 660
 aatgacatcg catggatctg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggctgacgg cgggtatacc gcacagtga 759

<210> 124
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 124
 Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Val Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Arg Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Ile Arg Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Glu Gly Leu Val Gly Asp Pro Thr Leu Gly Ala Tyr Asn Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Lys Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Phe Gly Glu Pro Asn Asp Ile Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 125
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 125
 atgaccgatac gtcgaaggg caaagtagcc atcgtaacg gcgggactct ggggttggt 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttatc tggtcgccac 120
 gcggatgtag gtgaaaaggc cgccaaatca atcgcgggca ctgatgtat tgcgtttgc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttggcccggt ttacgaccgt cgtgaacaat gcagggatg cagtttccaa aagcgttgaa 300
 gacactacca cggaggaatg gcgtaaactg ctgtccgta atctggatgg tgttttttc 360
 ggcacccgct tgggcattca gcgcatgaaa aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtggtt ctaggcgat ccgacgctgg ggcatacaa cgcttccaag 480
 gggcggtac gtatcatgtc gaaaagcgca gcgtggat gcgcactgaa ggactacgat 540
 gtgcgtgtca acacagtaca tccgggctat atcaagacct cgctggtcga tgatctggaa 600
 ggtgctgagg aatgatgtc acagcgtacg aaaacccta tgggccacat tggcgaaccg 660
 aatgacatcg catggatctg tgtgtacctg gcactgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 126
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 126
 Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Thr
 1 5 10 15
 Leu Gly Val Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Asn Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 127
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 127

376247016

atgaccgatac gtcgaaggg caaagtagcc atcgaaccg gcggggctct gggtatcggg 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggtcgccac 120
 gcggatgtag gtaaaaaggc cgccaaatca atcgccggca ctgatgtgt tgccttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttccgcccgg ttacgaccgt cgtgaacaat gcagggattg cagttccaa aagcgttgaa 300
 gacactacca cggaggaatg gcgtaaactg ctgtccgita atctggatgg tgttttttc 360
 ggcaccgcgc tgggcattca gcgcatgaaa aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtggtgt cgtaggcgat ccgacgctgg gggcatacaa cgcttccaag 480
 ggggcgggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
 gtgcgigtca acacagtaca tccgggctat atcaagaccc cgctggctga tgatctgaa 600
 ggtgctgagg aaatgatgtc acagcgtagc aaaaccccta tgggccacat tggcgaaccg 660
 aatgacatcg catggatctg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
 gcagaatttg tggtcgacgg cgggtatacc gcacagtga 759

<210> 128
 <211> 252
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 128
 Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Ala
 1 5 10 15
 Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
 20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Val Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Asn Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 129
 <211> 759
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Variant of L. kefir

<400> 129
 atgaccgatac gtcgaaggg caaagtagcc atcgaaccg gcggggctct gggtatcggg 60
 ttggcaatcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggtcgccac 120
 gcggatgtag gtaaaaaggc cgccaaatca atcgccggca ctgatgtat tgccttgtc 180
 cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
 ttccgcccgg ttacgaccgt cgtgaacaat gcagggattg cagttccaa aagcgttgaa 300
 gacactacca cggaggaatg gcgtaaactg ctgtccgita atctggatgg tgttttttc 360
 ggcaccgcgc tgggcattca gcgcatgaaa aataaaggct tgggcgctag catcatcaat 420
 atgagcagta tcagtggtgt cgtaggcgac ccgacgttag gggcatacaa cgcttccaag 480
 ggggcgggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540

376247016

gtgcgtgtca acacagtaca tccgggtat atcaagaccc cgtggtcga tgaatggaa 600
gggtgctagg aaatgatgc acagcgtacg aaaaccccta tgggccacat tggcgaaccg 660
aatgacatcg catggatcg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 130
<211> 252
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 130
Met Thr Asp Arg Leu Lys Gly Lys Val Ala Ile Val Thr Gly Gly Ala
1 5 10 15
Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala
20 25 30
Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
35 40 45
Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
50 55 60
Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
65 70 75 80
Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
85 90 95
Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
100 105 110
Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
115 120 125
Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
130 135 140
Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Asn Ala Ser Lys
145 150 155 160
Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
165 170 175
Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
180 185 190
Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
195 200 205
Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
210 215 220
Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
225 230 235 240
Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
245 250

<210> 131
<211> 759
<212> DNA
<213> Artificial Sequence

<220>
<223> Variant of L. kefir

<400> 131
atgaccgatc gtctgaaggc cagagtagcc atcgtaaccg gcgggactct gggatcggt 60
ttggcattcg ccgataaatt ttagaggag ggtgcgaaag tagttattac tggctgcac 120
gcggaigtac gtgaaaaggc cgccaaatca atcgcgcgca ctgatgttat tgcctttgtc 180
cagcaccgat catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
ttcggccggg ttacgaccgt cgtgaacaat gcagggatta cagcttccaa aagcgttgaa 300
gacactacca cggaggaatg gcataaactg ctgtccgtta atctggatgg tgttttttc 360
ggcaccgcgc tgggcattca gcgcataaaa aataaaggct tgggcgctag catcatcaat 420
atgagcagta ttgaggggtt cgtaggcgat ccgacgctgg gggcatacag cgcttccaag 480
ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
gtgcgtgtca acacagtaca tccgggtat atcaagaccc cgtggtcga tgaatggaa 600
gggtgctagg aaatgatgc acagcgtacg aaaaccccta tgggccacat tggcgaaccg 660
aatgacatcg catggatcg tgtgtacctg gcatctgacg aatcgaaatt tgcgacgggt 720
gcagaatttg tggcgacgg cgggtatacc gcacagtga 759

<210> 132
<211> 252
<212> PRT

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 132

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Met Thr Asp Arg Leu Lys Gly Arg Val Ala Ile Val Thr Gly Gly Thr
1      5      10     15
Leu Gly Ile Gly Leu Ala Phe Ala Asp Lys Phe Val Glu Glu Gly Ala
20     25     30
Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
35     40     45
Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
50     55     60
Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
65     70     75     80
Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Ala Ser
85     90     95
Lys Ser Val Glu Asp Thr Thr Thr Glu Trp His Lys Leu Leu Ser
100    105    110
Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
115    120    125
Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
130    135    140
Glu Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Ser Ala Ser Lys
145    150    155    160
Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
165    170    175
Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
180    185    190
Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
195    200    205
Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
210    215    220
Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
225    230    235    240
Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
245    250

```

<210> 133

<211> 759

<212> DNA

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 133

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atgacatc gtctgaagag caaagtagcc atcgtaacgg gcgggactct gggatcgggt 60
ttggcaatcg ccgataaatt ttagaggagg ggtgcgaaag tagttattac tggcgcac 120
gcgatgtag gtgaaaaggc cgccaaatca atcgccggta ctgatgttat togtttgtc 180
cagcacgatg catccgatga agcaggctgg acgaaactgt tcgacaccac cgaggaggca 240
ttcgcccggt ttacgacagt cgtgaacaat cgagggatta ccgtttcaa aagcgttgaa 300
gacactacca cggaggaatg gcacaaacta ctgtccgtta atctggatag tgttttttc 360
ggcaccgcgt tgggcattca ggcgatgaag aataaaggct tgggcgctag catcatcaat 420
atgagcagta tcagtgggtt cgtaggcgat ccgacgctgg gggcatacac tgctccaag 480
ggggcggtac gtatcatgtc gaaaagcgca gcgctggatt gcgcactgaa ggactacgat 540
gtcgtgtgta acacagtaca tccgggctat atcaagaccc cgctggtcga tgaictggaa 600
ggtgctgagg aaatgatgtc acagcgtacg aaaaccoccta tgggcacat tggogaaccg 660
aatgacgtgg catggtatctg tgtgtacctg gcactgacg aatcgaaatt tgcgacgggt 720
gcagaatttg tggtcgacgg cgggtatacc gcacagtga 759

```

<210> 134

<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> Variant of L. kefir

<400> 134

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Met Thr Tyr Arg Leu Lys Ser Lys Val Ala Ile Val Thr Gly Gly Thr
1      5      10     15
Leu Gly Ile Gly Leu Ala Ile Ala Asp Lys Phe Val Glu Glu Gly Ala

```

20 25 30
 Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
 35 40 45
 Lys Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
 50 55 60
 Ser Asp Glu Ala Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
 65 70 75 80
 Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Thr Val Ser
 85 90 95
 Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp His Lys Leu Leu Ser
 100 105 110
 Val Asn Leu Asp Ser Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
 115 120 125
 Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
 130 135 140
 Ser Gly Phe Val Gly Asp Pro Thr Leu Gly Ala Tyr Thr Ala Ser Lys
 145 150 155 160
 Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
 165 170 175
 Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
 180 185 190
 Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
 195 200 205
 Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Val Ala
 210 215 220
 Trp Ile Cys Val Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Gly Tyr Thr Ala Gln
 245 250

<210> 135

<211> 1041

<212> DNA

<213> Artificial Sequence

<220>

<223> Codon optimized ADH-SB from *S. salmonicolor*

<400> 135

atgaaggcac tgcagtaccg caccatcggc gccccgcccg aggtctgcac cgtcccgac 60
 ccggagccgg gccccggcca ggtgctgttg aaggtgaccg cggccggagt ctgccactcc 120
 gacatcgccg tgatgagctg gcccgcagag ggcttcccgt acgagctgcc gctcacctcc 180
 ggccacgagg gctgcggcac cgtggccgcg ctccggcccg gggtagcggg gctcggccag 240
 ggcgacgagg tggccgtgta cgggcccctg ggctgcggca cctgcgcca gtcgcccagg 300
 ggcaaggaga actactgctt gcgcgccgac gagctgggca tccgtccgcc ggggctcggg 360
 cgtccggggt ccatggccga gtacctgctg atcgacgacc cccggcacct ggtcccgtg 420
 gacgggctcg acccggtcgc ggcggcgccg ctacccgacg cggactgac gccgtaccac 480
 gcgatcaagc ggtcgtgccc caagctggtc cccggctcca ccgcggtggt catcggcacc 540
 ggtggtctcg gccacgtcgc catccagctg ctgcgcgcc tgacgtcgc cggggtggtc 600
 gcccggagc tcagcgagga gaagctgccc ctgcgccgtg cggtagggcg gcacgaggcg 660
 gtctgtcgg acgcgaaggc cgcggacgcg gtgcgcgaga tcaccggcgg tctcgggtcc 720
 gaggccgtgt tcgactctgt cggcgtggcg cccaccgtgc agaccgccc agccgtcgcg 780
 gccgtcagg gcatgtcac cctggtcggc atcgccggcg gatcgtgcc cgtcggcttc 840
 ggcatgctgc cgttcagggt gtccgtcaac gcccctact ggggcagccg cagcgagctg 900
 accgaggtgc tgaacctggc ccgctccggt gccgtgtcgg tgcacaccga gacgtactcc 960
 ctggacgacg ccccgctcgc ctacgagcgg ctgcacgagg gcagggtcaa cggccgcgcg 1020
 gtgactctgc cccacggctg a 1041

<210> 136

<211> 346

<212> PRT

<213> Artificial Sequence

<220>

<223> ADH-SB from *S. salmonicolor*

<400> 136

Met Lys Ala Leu Gln Tyr Arg Thr Ile Gly Ala Pro Pro Glu Val Val
 1 5 10 15
 Thr Val Pro Asp Pro Glu Pro Gly Pro Gly Gln Val Leu Leu Lys Val
 20 25 30
 Thr Ala Ala Gly Val Cys His Ser Asp Ile Ala Val Met Ser Trp Pro
 35 40 45
 Ala Glu Gly Phe Pro Tyr Glu Leu Pro Leu Thr Leu Gly His Glu Gly
 50 55 60

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Val Gly Thr Val Ala Ala Leu Gly Ala Gly Val Thr Gly Leu Ala Glu
 65 70 75 80
 Gly Asp Ala Val Ala Val Tyr Gly Pro Trp Gly Cys Gly Thr Cys Ala
 85 90 95
 Lys Cys Ala Glu Gly Lys Glu Asn Tyr Cys Leu Arg Ala Asp Glu Leu
 100 105 110
 Gly Ile Arg Pro Pro Gly Leu Gly Arg Pro Gly Ser Met Ala Glu Tyr
 115 120 125
 Leu Leu Ile Asp Asp Pro Arg His Leu Val Pro Leu Asp Gly Leu Asp
 130 135 140
 Pro Val Ala Ala Val Pro Leu Thr Asp Ala Gly Leu Thr Pro Tyr His
 145 150 155 160
 Ala Ile Lys Arg Ser Leu Pro Lys Leu Val Pro Gly Ser Thr Ala Val
 165 170 175
 Val Ile Gly Thr Gly Gly Leu Gly His Val Ala Ile Gln Leu Leu Arg
 180 185 190
 Ala Leu Thr Ser Ala Arg Val Val Ala Leu Asp Val Ser Glu Glu Lys
 195 200 205
 Leu Arg Leu Ala Arg Ala Val Gly Ala His Glu Ala Val Leu Ser Asp
 210 215 220
 Ala Lys Ala Ala Asp Ala Val Arg Glu Ile Thr Gly Gly Leu Gly Ala
 225 230 235 240
 Glu Ala Val Phe Asp Phe Val Gly Val Ala Pro Thr Val Gln Thr Ala
 245 250 255
 Gly Ala Val Ala Ala Val Glu Gly Asp Val Thr Leu Val Gly Ile Gly
 260 265 270
 Gly Gly Ser Leu Pro Val Gly Phe Gly Met Leu Pro Phe Glu Val Ser
 275 280 285
 Val Asn Ala Pro Tyr Trp Gly Ser Arg Ser Glu Leu Thr Glu Val Leu
 290 295 300
 Asn Leu Ala Arg Ser Gly Ala Val Ser Val His Thr Glu Thr Tyr Ser
 305 310 315 320
 Leu Asp Asp Ala Pro Leu Ala Tyr Glu Arg Leu His Glu Gly Arg Val
 325 330 335
 Asn Gly Arg Ala Val Ile Leu Pro His Gly
 340 345

<210> 137
 <211> 1032
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Codon optimized ADH-SC from *S. coelicolor*

<400> 137
 atggcaaaaga tgcacaacgc agttctgccg gaggggttctc tgggtctggt caccggcgcg 60
 aacggctttg tgcctagcca tgggtcgaa caactgctgg aacacggcta taagggtgoc 120
 ggcactgctc gctctgcctc caaactggcg aacctgcaga aacgttgga cgccaaatac 180
 cctggctgtt tgcagactgc cgttgttgaa gacatgctga agcagggtgc atatgatgaa 240
 gttattaaag gcgcggcagg tgcgcccac atcgctccg tggcagctt ttctaacaaa 300
 tatgatgagg tggtaactcc tgcgatcgtt ggcacgctga atgccctgcg tgcgcagct 360
 gctacgctt cctgaaacg tttgtgctg accagcagca ctgttctgc actgaticca 420
 aaacctaacg tcgaaggat ttatctggat gagaagagct ggaacctgga aagcattgat 480
 aaggctaaaa cctgcctga atctgatccg cagaaaagcc tgggtgcta cgccgcaagc 540
 aaaacggaag cggaactggc tgcctggaaa tcatggagc aaaacaaacc gcactttact 600
 ctgaatgcg ttcgccaata ctacactatc ggtaccatt ttgaccaga aacccaatcc 660
 ggttccactt ccgctggat gatgtctctg ttcaatggcg aagtatctcc ggcactggcg 720
 ctgatgcgcg cgcagtacta tgcctctgca gttgatatcg gtcgtctgca cctgggttgt 780
 ctggttctgc cgcaaatcga acgcccgtct gttacggca ccgcaggcac ctttgattgg 840
 aacaccgttc tggcgacctt ccgtaaactg tatccgtcca agacgttccc ggctgacttt 900
 ccggatcagg gccaggatct gtccaaattt gataccgcc cgagcctgga gattctgaaa 960
 tccctgggcc gccctggctg gcgtagcatc gaggaatcta tcaaagatct ggtgggttcc 1020
 gagaccgct aa 1032

<210> 138
 <211> 343
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> ADH-SC from *S. coelicolor*

<400> 138

376247016

Met Ala Lys Ile Asp Asn Ala Val Leu Pro Glu Gly Ser Leu Val Leu
 1 5 10 15
 Val Thr Gly Ala Asn Gly Phe Val Ala Ser His Val Val Glu Gln Leu
 20 25 30
 Leu Glu His Gly Tyr Lys Val Arg Gly Thr Ala Arg Ser Ala Ser Lys
 35 40 45
 Leu Ala Asn Leu Gln Lys Arg Trp Asp Ala Lys Tyr Pro Gly Arg Phe
 50 55 60
 Glu Thr Ala Val Val Glu Asp Met Leu Lys Gln Gly Ala Tyr Asp Glu
 65 70 75 80
 Val Ile Lys Gly Ala Ala Gly Val Ala His Ile Ala Ser Val Val Ser
 85 90 95
 Phe Ser Asn Lys Tyr Asp Glu Val Val Thr Pro Ala Ile Gly Gly Thr
 100 105 110
 Leu Asn Ala Leu Arg Ala Ala Ala Thr Pro Ser Val Lys Arg Phe
 115 120 125
 Val Leu Thr Ser Ser Thr Val Ser Ala Leu Ile Pro Lys Pro Asn Val
 130 135 140
 Glu Gly Ile Tyr Leu Asp Glu Lys Ser Trp Asn Leu Glu Ser Ile Asp
 145 150 155 160
 Lys Ala Lys Thr Leu Pro Glu Ser Asp Pro Gln Lys Ser Leu Trp Val
 165 170 175
 Tyr Ala Ala Ser Lys Thr Glu Ala Glu Leu Ala Ala Trp Lys Phe Met
 180 185 190
 Asp Glu Asn Lys Pro His Phe Thr Leu Asn Ala Val Leu Pro Asn Tyr
 195 200 205
 Thr Ile Gly Thr Ile Phe Asp Pro Glu Thr Gln Ser Gly Ser Thr Ser
 210 215 220
 Gly Trp Met Met Ser Leu Phe Asn Gly Glu Val Ser Pro Ala Leu Ala
 225 230 235 240
 Leu Met Pro Pro Gln Tyr Tyr Val Ser Ala Val Asp Ile Gly Leu Leu
 245 250 255
 His Leu Gly Cys Leu Val Leu Pro Gln Ile Glu Arg Arg Arg Val Tyr
 260 265 270
 Gly Thr Ala Gly Thr Phe Asp Trp Asn Thr Val Leu Ala Thr Phe Arg
 275 280 285
 Lys Leu Tyr Pro Ser Lys Thr Phe Pro Ala Asp Phe Pro Asp Gln Gly
 290 295 300
 Gln Asp Leu Ser Lys Phe Asp Thr Ala Pro Ser Leu Glu Ile Leu Lys
 305 310 315 320
 Ser Leu Gly Arg Pro Gly Trp Arg Ser Ile Glu Glu Ser Ile Lys Asp
 325 330 335
 Leu Val Gly Ser Glu Thr Ala
 340

<210> 139

<211> 1056

<212> DNA

<213> Artificial Sequence

<220>

<223> Codon optimized ADH-TB from T. Brockii

<400> 139

atgaaaggct tgcctatgct gagcatggc aaagtgggtt ggattgaaaa agaaaaaccg 60
 gcgccaggcc cgttcgatgc aattgtgcgc cctctggcag tagcgccgtg taccagcgat 120
 attcatactg tgtttgaagg tgccattggc gagcgtcaca atatgattct gggccatgaa 180
 gccgttggtg aagttgttga ggttggcagc gaagtgaagg attcaaaacc ggccgatcgc 240
 gttgtcgttc cagcgattac cccggattgg cgcaccagcg aagtcagcg cggctaccat 300
 cagcactctg gcgcgatcgt ggccggcgtg aaattcagca atgtaaagga tgggtgttc 360
 ggigaatttt ttacgttaa cgaacgagac atgaatctgg cgcacctgcc gaaagaaatc 420
 ccgctggaag cagcggttat gattccggt atgatgacca cgggttttca cggcgagag 480
 ctggcggaca tgaactggg cgtacggta gccgtactgg gcatcggtcc ggtgggctcg 540
 atggcagttg caggcgctaa gctgcggcgc gcaggtcgta ttatgccgt tggttctcgc 600
 ccggtgtgtg tggacgcgc taagtattat ggtgcaacgg acattgtcaa ttacaaggac 660
 ggcccaattg aatctcagat catgaacctg acggaaggta aaggcgttga cggcgagatt 720
 atcgctggcg gcaacgcga catcatggcg accgcagta aaatcgtcaa gccaggtggt 780
 aatattgcta acgtcaacta ctgcggcga ggtgaggttc tgcctgtccc acgtctggaa 840
 tgggtgtggt gtagggcaca taaaaccatt aaaggtggcc tggccagg cgccgctcg 900
 cgtatggaac gccatgacga tctgtcttc tacaacgcg tggatctag caaactggtg 960
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 20 25 30
 Ala Val Ala Pro Cys Thr Ser Asp Ile His Thr Val Phe Glu Gly Ala
 35 40 45
 Ile Gly Glu Arg His Asn Met Ile Leu Gly His Glu Ala Val Gly Glu
 50 55 60
 Val Val Glu Val Gly Ser Glu Val Lys Asp Phe Lys Pro Gly Asp Arg
 65 70 75 80
 Val Val Val Pro Ala Ile Thr Pro Asp Trp Arg Thr Ser Glu Val Gln
 85 90 95
 Arg Gly Tyr His Gln His Ser Gly Gly Met Leu Ala Gly Trp Lys Phe
 100 105 110
 Ser Asn Val Lys Asp Gly Val Phe Gly Glu Phe Phe His Val Asn Asp
 115 120 125
 Ala Asp Met Asn Leu Ala His Leu Pro Lys Glu Ile Pro Leu Glu Ala
 130 135 140
 Ala Val Met Ile Pro Asp Met Met Thr Thr Gly Phe His Gly Ala Glu
 145 150 155 160
 Leu Ala Asp Ile Glu Leu Gly Ala Thr Val Ala Val Leu Gly Ile Gly
 165 170 175
 Pro Val Gly Leu Met Ala Val Ala Gly Ala Lys Leu Arg Gly Ala Gly
 180 185 190
 Arg Ile Ile Ala Val Gly Ser Arg Pro Val Cys Val Asp Ala Ala Lys
 195 200 205
 Tyr Tyr Gly Ala Thr Asp Ile Val Asn Tyr Lys Asp Gly Pro Ile Glu
 210 215 220
 Ser Gln Ile Met Asn Leu Thr Glu Gly Lys Gly Val Asp Ala Ala Ile
 225 230 235 240
 Ile Ala Gly Gly Asn Ala Asp Ile Met Ala Thr Ala Val Lys Ile Val
 245 250 255
 Lys Pro Gly Gly Thr Ile Ala Asn Val Asn Tyr Phe Gly Glu Gly Glu
 260 265 270
 Val Leu Pro Val Pro Arg Leu Glu Trp Gly Cys Gly Met Ala His Lys
 275 280 285
 Thr Ile Lys Gly Gly Leu Cys Pro Gly Gly Arg Leu Arg Met Glu Arg
 290 295 300
 Leu Ile Asp Leu Val Phe Tyr Lys Arg Val Asp Pro Ser Lys Leu Val
 305 310 315 320
 Thr His Val Phe Arg Gly Phe Asp Asn Ile Glu Lys Ala Phe Met Leu
 325 330 335
 Met Lys Asp Lys Pro Lys Asp Leu Ile Lys Pro Val Val Ile Leu Ala
 340 345 350

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 gctgatgtag gtgaaaaagc tgccagatca atcgcgcgca cagacgttat ccgtttgtc 180
 caacacgatg ctctgatga aaccggtcgg actaagttgt ttgatacgac tgaagaagca 240
 ttggcccag ttaccacggt tgcacaat gccggaattg cggtcagcaa gagtgttgaa 300
 gataccacaa ctgaagaatg gcgcaagctg ctctcagta acttgatgg tgtctcttc 360
 ggtaccgcgc ttggaatcca acgtatgaag aataaaggac tcggagcatc aatcatcaat 420
 atgtcatcta tcgaaggttt tgttggtgat ccagctctgg gtgcatacaa cgcttcaaaa 480

376247016

gggtgctgta gaattatgtc taaatcagct gccttggatt gcgctttgaa ggactacgat 540
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ggggcagaag aaatgatgtc acagcggacc aagacaccaa tgggtcatat cggatgaacct 660
aacgatatcg ctggatctg tgtttacctg gcactcgaag aatctaaatt tgccactggt 720
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Lys Val Val Ile Thr Gly Arg His Ala Asp Val Gly Glu Lys Ala Ala
35 40 45
Arg Ser Ile Gly Gly Thr Asp Val Ile Arg Phe Val Gln His Asp Ala
50 55 60
Ser Asp Glu Thr Gly Trp Thr Lys Leu Phe Asp Thr Thr Glu Glu Ala
65 70 75 80
Phe Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Ala Val Ser
85 90 95
Lys Ser Val Glu Asp Thr Thr Thr Glu Glu Trp Arg Lys Leu Leu Ser
100 105 110
Val Asn Leu Asp Gly Val Phe Phe Gly Thr Arg Leu Gly Ile Gln Arg
115 120 125
Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
130 135 140
Glu Gly Phe Val Gly Asp Pro Ala Leu Gly Ala Tyr Asn Ala Ser Lys
145 150 155 160
Gly Ala Val Arg Ile Met Ser Lys Ser Ala Ala Leu Asp Cys Ala Leu
165 170 175
Lys Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Lys
180 185 190
Thr Pro Leu Val Asp Asp Leu Glu Gly Ala Glu Glu Met Met Ser Gln
195 200 205
Arg Thr Lys Thr Pro Met Gly His Ile Gly Glu Pro Asn Asp Ile Ala
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Lys Val Val Ile Thr Gly Arg His Xaa Asp Val Gly Xaa Lys Ala Ala
 35     40     45
Xaa Ser Ile Gly Gly Thr Asp Val Xaa Arg Phe Xaa Gln His Asp Xaa
 50     55     60
Ser Asp Glu Thr Gly Trp Thr Xaa Leu Phe Asp Thr Thr Glu Glu Ala
 65     70     75     80
Xaa Gly Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Xaa Xaa Xaa
 85     90     95
Xaa Ser Val Glu Asp Thr Thr Thr Glu Xaa Trp Xaa Lys Leu Xaa Ser
100    105    110
Val Asn Leu Asp Xaa Val Phe Phe Gly Thr Arg Leu Gly Xaa Xaa Arg
115    120    125
Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
130    135    140

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 145 150 155 160
 Gly Ala Xaa Arg Ile Met Ser Lys Ser Ala Ala Leu Xaa Cys Ala Leu
 165 170 175
 Xaa Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Xaa
 180 185 190
 Thr Xaa Leu Val Asp Xaa Leu Xaa Gly Ala Glu Glu Met Xaa Ser Xaa
 195 200 205
 Arg Xaa Xaa Thr Pro Xaa Gly His Xaa Gly Glu Pro Asn Asp Xaa Ala
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 Trp Xaa Cys Xaa Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
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Lys Val Met Ile Thr Gly Arg His Xaa Asp Val Gly Xaa Lys Ala Ala
35     40     45
Xaa Ser Val Gly Thr Pro Asp Gln Xaa Gln Phe Xaa Gln His Asp Xaa
50     55     60
Ser Asp Glu Asp Gly Trp Thr Xaa Leu Phe Asp Ala Thr Glu Lys Ala
65     70     75     80
Phe Xaa Pro Val Ser Thr Leu Val Asn Asn Ala Gly Ile Xaa Xaa Xaa
85     90     95
Xaa Ser Val Glu Glu Thr Thr Ala Xaa Trp Xaa Lys Leu Xaa Ala
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Val Asn Leu Asp Xaa Val Phe Phe Gly Thr Arg Leu Gly Xaa Xaa Arg
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Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile
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Gly Ala Xaa Arg Ile Met Ser Lys Ser Ala Ala Leu Xaa Cys Ala Leu
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Xaa Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Xaa
180    185    190
Thr Xaa Leu Val Asp Xaa Leu Xaa Gly Ala Glu Glu Ala Xaa Ser Xaa
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Arg Xaa Xaa Thr Pro Xaa Gly His Xaa Gly Glu Pro Asn Asp Xaa Ala
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<223> Xaa is an aromatic, aliphatic, polar, or non-polar residue

<220> FEATURE
<221> MISC_FEATURE
<222> (152)..(152)
<223> Xaa is a polar, aliphatic or nonpolar residue

<220> FEATURE
<221> MISC_FEATURE
<222> (157)..(157)
<223> Xaa is a polar or acidic residue

<220> FEATURE
<221> MISC_FEATURE
<222> (163)..(163)
<223> Xaa is an aliphatic or nonpolar residue

<220> FEATURE
<221> MISC_FEATURE
<222> (173)..(173)
<223> Xaa is an acidic or non-polar residue

<220> FEATURE
<221> MISC_FEATURE
<222> (177)..(177)
<223> Xaa is a basic residue

<220> FEATURE
<221> MISC_FEATURE
<222> (192)..(192)
<223> Xaa is a basic or acidic residue

<220> FEATURE
<221> MISC_FEATURE
<222> (194)..(194)
<223> Xaa is a constrained, acidic, aliphatic, polar, or non-polar residue

<220> FEATURE
<221> MISC_FEATURE

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<222> (198)..(198)

<223> Xaa is an acidic or non-polar residue

<220> FEATURE

<221> MISC_FEATURE

<222> (200)..(200)

<223> Xaa is a constrained, acidic, or basic residue

<220> FEATURE

<221> MISC_FEATURE

<222> (206)..(206)

<223> Xaa is a polar or non-polar residue

<220> FEATURE

<221> MISC_FEATURE

<222> (208)..(208)

<223> Xaa is a polar, constrained, or basic residue

<220> FEATURE

<221> MISC_FEATURE

<222> (210)..(210)

<223> Xaa is a aliphatic, polar or non-polar residue

<220> FEATURE

<221> MISC_FEATURE

<222> (211)..(211)

<223> Xaa is a basic or acidic residue

<220> FEATURE

<221> MISC_FEATURE

<222> (214)..(214)

<223> Xaa is a nonpolar, aliphatic or polar residue

<220> FEATURE

<221> MISC_FEATURE

<222> (217)..(217)

<223> Xaa is an aromatic, aliphatic or non-polar residue

<220> FEATURE

<221> MISC_FEATURE

<222> (223)..(223)

<223> Xaa is an aliphatic or nonpolar residue

<220> FEATURE

<221> MISC_FEATURE

<222> (226)..(226)

<223> Xaa is an nonpolar or aliphatic residue

<220> FEATURE

<221> MISC_FEATURE

<222> (228)..(228)

<223> Xaa is an nonpolar or aliphatic residue

<400> 145

Met Thr Xaa Arg Leu Lys Xaa Lys Val Ala Xaa Val Thr Gly Gly Xaa

1 5 10 15

Leu Gly Xaa Gly Leu Ala Xaa Ala Asp Lys Phe Val Glu Glu Gly Ala

20 25 30

Lys Val Val Ile Thr Gly Arg His Xaa Asp Val Gly Xaa Lys Ala Ala

35 40 45

Xaa Ser Ile Gly Gly Thr Asp Val Xaa Arg Phe Xaa Gln His Asp Xaa

50 55 60

Ser Asp Glu Ala Gly Trp Thr Xaa Leu Phe Asp Thr Thr Glu Glu Ala

65 70 75 80

Phe Xaa Pro Val Thr Thr Val Val Asn Asn Ala Gly Ile Xaa Xaa Xaa

85 90 95

Xaa Ser Val Glu Asp Thr Thr Thr Glu Xaa Trp Xaa Lys Leu Xaa Ser

100 105 110

Val Asn Leu Asp Xaa Val Phe Phe Gly Thr Arg Leu Gly Xaa Xaa Arg

115 120 125

Met Lys Asn Lys Gly Leu Gly Ala Ser Ile Ile Asn Met Ser Ser Ile

130 135 140

Xaa Gly Xaa Val Gly Asp Pro Xaa Leu Gly Ala Tyr Xaa Ala Ser Lys

145 150 155 160

Gly Ala Xaa Arg Ile Met Ser Lys Ser Ala Ala Leu Xaa Cys Ala Leu

165 170 175

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Xaa Asp Tyr Asp Val Arg Val Asn Thr Val His Pro Gly Tyr Ile Xaa
 180 185 190
 Thr Xaa Leu Val Asp Xaa Leu Xaa Gly Ala Glu Glu Met Xaa Ser Xaa
 195 200 205
 Arg Xaa Xaa Thr Pro Xaa Gly His Xaa Gly Glu Pro Asn Asp Xaa Ala
 210 215 220
 Trp Xaa Cys Xaa Tyr Leu Ala Ser Asp Glu Ser Lys Phe Ala Thr Gly
 225 230 235 240
 Ala Glu Phe Val Val Asp Gly Tyr Thr Ala Gln
 245 250

<210> 146
 <211> 939
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> YDL ADH from *Saccharomyces cerevisiae*

<400> 146
 atgtctttt accaacagtt tttaacgctg aacaacggca ataaatccc ggcgattgcc 60
 atcatcggca ctggtacacg ttggtataaa aatgaagaaa ctgacgcgac cttctccaat 120
 agtctggttg aacaaatcgt gtatgcgttg aaactgcctg ggattatcca catcgacgcc 180
 gcggagattt atgcaccta cccggaagtg ggtaaacgac tgcctcgac cgaaaagcct 240
 cgtaacgca ttttctgac ggataaatai tctccgcaga ttaaatgag tgactccct 300
 gcggacgctc tggatttagc attgaagaaa atgggtacag attatgtga ttatatctg 360
 ttacattccc cgttgttc gaaggaagt aatggcttaa gcttagaaga ggcttgaaa 420
 gatatggagc agttatacaa aagtggtaaa gctaaaaaca tgggggttc caatttcgca 480
 gtgaagacc tgcaacgtat cctgaaagtc gctgaagta aacctcaggt caaccagatt 540
 gagttctctc cgttctgca aaaccaaaca ccaggcattt ataaattctg tcaggagcac 600
 gatatcctgg tggaagcata ttctccgctg ggcccgcctg agaagaaaac cgcgcaggat 660
 gacagccaac catTTTTga gtaogtcaaa gaattgagcg aaaaatacat caaatccgag 720
 gccagatca tctgcgctg ggtcactaaa cgcgggtgic tgccagtac cacccttca 780
 aagcctcagc gcattagcga tgctcagaac ctgtttctc tgcacctgac agcgggaagag 840
 gttgataaaa tcacggagct gggtctggaa catgaaccgc tgcgcctgta ctggaataaa 900
 ttgtatggca aatataacta cgcgcgccag aaagtgtaa 939

<210> 147
 <211> 312
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> YDL ADH from *Saccharomyces cerevisiae*

<400> 147
 Met Ser Phe His Gln Gln Phe Phe Thr Leu Asn Asn Gly Asn Lys Ile
 1 5 10 15
 Pro Ala Ile Ala Ile Ile Gly Thr Gly Thr Arg Trp Tyr Lys Asn Glu
 20 25 30
 Glu Thr Asp Ala Thr Phe Ser Asn Ser Leu Val Glu Gln Ile Val Tyr
 35 40 45
 Ala Leu Lys Leu Pro Gly Ile Ile His Ile Asp Ala Ala Glu Ile Tyr
 50 55 60
 Arg Thr Tyr Pro Glu Val Gly Lys Ala Leu Ser Leu Thr Glu Lys Pro
 65 70 75 80
 Arg Asn Ala Ile Phe Leu Thr Asp Lys Tyr Ser Pro Gln Ile Lys Met
 85 90 95
 Ser Asp Ser Pro Ala Asp Gly Leu Asp Leu Ala Leu Lys Lys Met Gly
 100 105 110
 Thr Asp Tyr Val Asp Leu Tyr Leu Leu His Ser Pro Phe Val Ser Lys
 115 120 125
 Glu Val Asn Gly Leu Ser Leu Glu Glu Ala Trp Lys Asp Met Glu Gln
 130 135 140
 Leu Tyr Lys Ser Gly Lys Ala Lys Asn Ile Gly Val Ser Asn Phe Ala
 145 150 155 160
 Val Glu Asp Leu Gln Arg Ile Leu Lys Val Ala Glu Val Lys Pro Gln
 165 170 175
 Val Asn Gln Ile Glu Phe Ser Pro Phe Leu Gln Asn Gln Thr Pro Gly
 180 185 190
 Ile Tyr Lys Phe Cys Gln Glu His Asp Ile Leu Val Glu Ala Tyr Ser
 195 200 205
 Pro Leu Gly Pro Leu Gln Lys Lys Thr Ala Gln Asp Asp Ser Gln Pro
 210 215 220

376247016

Phe Phe Glu Tyr Val Lys Glu Leu Ser Glu Lys Tyr Ile Lys Ser Glu
 225 230 235 240
 Ala Gln Ile Ile Leu Arg Trp Val Thr Lys Arg Gly Val Leu Pro Val
 245 250 255
 Thr Thr Ser Ser Lys Pro Gln Arg Ile Ser Asp Ala Gln Asn Leu Phe
 260 265 270
 Ser Phe Asp Leu Thr Ala Glu Glu Val Asp Lys Ile Thr Glu Leu Gly
 275 280 285
 Leu Glu His Glu Pro Leu Arg Leu Tyr Trp Asn Lys Leu Tyr Gly Lys
 290 295 300
 Tyr Asn Tyr Ala Ala Gln Lys Val
 305 310

<210> 148
 <211> 1047
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> ADH from Rhodococcus erythropolis

<400> 148
 atgaaagcca ttcagtacac tcgtatcggt gcggaaccag aactgactga aatccccaag 60
 ccggaaccgg gcccgggcga agtactgctg gaagtcacgg cagctggcgt gtgccattcc 120
 gatgatttca ttatgtctct gccggaagaa cagtacacct acggcctgcc cctgaccctg 180
 ggctatgaag gtgctggtaa agttgccgca gttggcgaag gtgtgaagg gttggatatt 240
 ggcaccaatg tggttgtgta cggcccatgg ggttgggca actgttggca ttgcagtcag 300
 ggcttggaga actatgtctc cgtgctgcag gaactgggta ttaaccggcc tggctgggt 360
 gctcgggggg cttggcaga atttatgatt gtcgactcac cagtcattt ggtcccgatt 420
 ggcgatttag accctgttaa aactgttccg ttgactgatg cgggcctgac cccataccat 480
 gcaatcaaac gctccctgcc gaaactgcgc ggcggctctt atgcagtagt gatcggtaag 540
 ggtggcctgg gccacgtggc tatccaactg ctgctgctt tatctgtgc aacggtgatc 600
 gccttggacg ttctgccga taaactggaa ctggctacca aagtcggcgc acatgaagta 660
 gtctgtctg ataaagatgc agcggagaat gtgcgtaaaa ttactgtag ccaaggtgca 720
 gcttgggtgt tggatttgt ggcctatcag cctaccattg acaccgccat ggcagtgccg 780
 ggctggggct ctgacgtcac cattgtgtgt atcggtgatg gccaggcaca tgcgaaagt 840
 ggcttctcc agagtcctta tgaggcatcg gttacggtag cttattgggg cgctcgtaat 900
 gaactgatcg aattgatcga tctggcgcgt cctgttattt tgcacattgc cgttgagacc 960
 ttctttggg ataattgtgc agaggcctat cgtcgctcgt ctcggggcac actgtcaggc 1020
 cgtcggttag tcgtcccggt cctgtaa 1047

<210> 149
 <211> 348
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> ADH from Rhodococcus erythropolis

<400> 149
 Met Lys Ala Ile Gln Tyr Thr Arg Ile Gly Ala Glu Pro Glu Leu Thr
 1 5 10 15
 Glu Ile Pro Lys Pro Glu Pro Gly Pro Gly Glu Val Leu Leu Glu Val
 20 25 30
 Thr Ala Ala Gly Val Cys His Ser Asp Asp Phe Ile Met Ser Leu Pro
 35 40 45
 Glu Glu Gln Tyr Thr Tyr Gly Leu Pro Leu Thr Leu Gly His Glu Gly
 50 55 60
 Ala Gly Lys Val Ala Ala Val Gly Glu Gly Val Glu Gly Leu Asp Ile
 65 70 75 80
 Gly Thr Asn Val Val Tyr Gly Pro Trp Gly Cys Gly Asn Cys Trp
 85 90 95
 His Cys Ser Gln Gly Leu Glu Asn Tyr Cys Ser Arg Ala Gln Glu Leu
 100 105 110
 Gly Ile Asn Pro Pro Gly Leu Gly Ala Pro Gly Ala Leu Ala Glu Phe
 115 120 125
 Met Ile Val Asp Ser Pro Arg His Leu Val Pro Ile Gly Asp Leu Asp
 130 135 140
 Pro Val Lys Thr Val Pro Leu Thr Asp Ala Gly Leu Thr Pro Tyr His
 145 150 155 160
 Ala Ile Lys Arg Ser Leu Pro Lys Leu Arg Gly Gly Ser Tyr Ala Val
 165 170 175
 Val Ile Gly Thr Gly Gly Leu Gly His Val Ala Ile Gln Leu Leu Arg
 180 185 190
 His Leu Ser Ala Ala Thr Val Ile Ala Leu Asp Val Ser Ala Asp Lys

195 200 205
 Leu Glu Leu Ala Thr Lys Val Gly Ala His Glu Val Val Leu Ser Asp
 210 215 220
 Lys Asp Ala Ala Glu Asn Val Arg Lys Ile Thr Gly Ser Gln Gly Ala
 225 230 235 240
 Ala Leu Val Leu Asp Phe Val Gly Tyr Gln Pro Thr Ile Asp Thr Ala
 245 250 255
 Met Ala Val Ala Gly Val Gly Ser Asp Val Thr Ile Val Gly Ile Gly
 260 265 270
 Asp Gly Gln Ala His Ala Lys Val Gly Phe Phe Gln Ser Pro Tyr Glu
 275 280 285
 Ala Ser Val Thr Val Pro Tyr Trp Gly Ala Arg Asn Glu Leu Ile Glu
 290 295 300
 Leu Ile Asp Leu Ala His Ala Gly Ile Phe Asp Ile Ala Val Glu Thr
 305 310 315 320
 Phe Ser Leu Asp Asn Gly Ala Glu Ala Tyr Arg Arg Leu Ala Ala Gly
 325 330 335
 Thr Leu Ser Gly Arg Ala Val Val Val Pro Gly Leu
 340 345

<210> 150
 <211> 1047
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> YGL ADH from *Saccharomyces cerevisiae*

<400> 150
 atgaccacgg aaaaaacgt agtgttcgtg tcaggcgcca ccggttttat tgctctgcac 60
 gtggtagatg acctgctgaa aactggttac aaagtaattg gtccgggtcg ttctcaggaa 120
 aaaaatgacg gtttgctgaa gaagttcaag tccaaccoga atctgagcat ggaaattgtg 180
 gaagatattg cggcaccaaa cgcttcgat aaagtattcc agaaacatgg taaagaaatt 240
 aaagtgttcc tgcatacgc giccccggtc cattcaaca ctaccgattt cgaataaacg 300
 ttactgatcc cggcggttaa cggtaacaaa tctatttgg aagcaattaa gaactatgcc 360
 gcagacacgg tggaaaaagt ggttattact tcatctgttg ccgcgttggc ctctcgggt 420
 gataagaaag ataccagctt cgtggttaac gaagaatcct ggaataaaga cactcgggaa 480
 tctgtcagg cgaatgctgt gtccggttat tgcggttcta aaaaattcgc agagaaaaacg 540
 ggtgtggact tcttgaaga aaaccagagc agcattaaat ttactctgtc cagattaac 600
 ccaggtctcg ttttgggcc gcagctgttc gccgactcct tgcgcaatgg tattaactct 660
 agcagtgcca ttattgcgaa cctggtgtcg tataaattag gggataacti ctacaattat 720
 agcggccctg ttatcgactt cgtgacgtt tccaagctc atctgctggc attgagaaa 780
 cctgaatgcg ccggtcagcg cctgtttctg tgcgaggata tgttctgttc ccaggaagcc 840
 ctggacattc tgaacgaaga atttcacag ctgaaggcca agatcgcaac gggcgaacct 900
 ggcagcggct cgacctctct gactaaaaat tgttgcaaat gcgacaatcg taaaactaaa 960
 aactgtcgg gcttccagtt caacaaattc cgcgactgca ttgtcgatc tgcgtcccag 1020
 ttgtggaag tgcaaagcaa aagctaa 1047

<210> 151
 <211> 348
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> YGL ADH from *Saccharomyces cerevisiae*

<400> 151
 Met Thr Thr Glu Lys Thr Val Val Phe Val Ser Gly Ala Thr Gly Phe
 1 5 10 15
 Ile Ala Leu His Val Val Asp Asp Leu Leu Lys Thr Gly Tyr Lys Val
 20 25 30
 Ile Gly Ser Gly Arg Ser Gln Glu Lys Asn Asp Gly Leu Leu Lys Lys
 35 40 45
 Phe Lys Ser Asn Pro Asn Leu Ser Met Glu Ile Val Glu Asp Ile Ala
 50 55 60
 Ala Pro Asn Ala Phe Asp Lys Val Phe Gln Lys His Gly Lys Glu Ile
 65 70 75 80
 Lys Val Val Leu His Ile Ala Ser Pro Val His Phe Asn Thr Thr Asp
 85 90 95
 Phe Glu Lys Asp Leu Leu Ile Pro Ala Val Asn Gly Thr Lys Ser Ile
 100 105 110
 Leu Glu Ala Ile Lys Asn Tyr Ala Ala Asp Thr Val Glu Lys Val Val
 115 120 125
 Ile Thr Ser Ser Val Ala Ala Leu Ala Ser Pro Gly Asp Met Lys Asp

130 135 140
 Thr Ser Phe Val Val Asn Glu Glu Ser Trp Asn Lys Asp Thr Trp Glu
 145 150 155 160
 Ser Cys Gln Ala Asn Ala Val Ser Ala Tyr Cys Gly Ser Lys Lys Phe
 165 170 175
 Ala Glu Lys Thr Ala Trp Asp Phe Leu Glu Glu Asn Gln Ser Ser Ile
 180 185 190
 Lys Phe Thr Leu Ser Thr Ile Asn Pro Gly Phe Val Phe Gly Pro Gln
 195 200 205
 Leu Phe Ala Asp Ser Leu Arg Asn Gly Ile Asn Ser Ser Ser Ala Ile
 210 215 220
 Ile Ala Asn Leu Val Ser Tyr Lys Leu Gly Asp Asn Phe Tyr Asn Tyr
 225 230 235 240
 Ser Gly Pro Phe Ile Asp Val Arg Asp Val Ser Lys Ala His Leu Leu
 245 250 255
 Ala Phe Glu Lys Pro Glu Cys Ala Gly Gln Arg Leu Phe Leu Cys Glu
 260 265 270
 Asp Met Phe Cys Ser Gln Glu Ala Leu Asp Ile Leu Asn Glu Glu Phe
 275 280 285
 Pro Gln Leu Lys Gly Lys Ile Ala Thr Gly Glu Pro Gly Ser Gly Ser
 290 295 300
 Thr Phe Leu Thr Lys Asn Cys Cys Lys Cys Asp Asn Arg Lys Thr Lys
 305 310 315 320
 Asn Leu Leu Gly Phe Gln Phe Asn Lys Phe Arg Asp Cys Ile Val Asp
 325 330 335
 Thr Ala Ser Gln Leu Leu Glu Val Gln Ser Lys Ser
 340 345

<210> 152

<211> 939

<212> DNA

<213> Artificial Sequence

<220>

<223> YPR ADH from *Saccharomyces cerevisiae*

<400> 152

atgccggcaa cgtaaaaaa cagcagtgct accttaaaat taaacacagg tgcgagcatt 60
 cctgtctcgg ggctcggcac ctggcgctct gtcgataaca acggctatca tagtgaatt 120
 gcggcgctga aagcggggta ccgtcataatc gatgctgcgg ccactatcti gaatgaagaa 180
 gaagtcggcc gtgcgatcaa ggactccggti gttcctcgtg aagaaattti tattaccacc 240
 aaactgtggg gcaccgaaca acgcgatcca gaagcagccc tgaacaaatc tctgaaacgt 300
 ctgggtctgg actatgtgga cctgtatcig atgcactggc cgttccctct gaaaacagac 360
 cgtgtactgt acggtaacgt cctgtgcatc ccgaccctgg aagatggcac cgtggacatc 420
 gataccaaag agtggaaatt tattaaaacc tgggaactga tgcaggaatt gccgaaaact 480
 ggtaagacca aagccgtcgg tgtgtccaat tticcataca acaatatcaa agaactgctg 540
 gaatcgccaa ataacaaggt cgttcagca accaatcaga tcgagattca tccgtgtcig 600
 ccgaggatgt aattaatcgc ctittgtaaa gaaaaaggca ttgtggtcga agcatatagc 660
 ccattcggct ccgttaacgc ccgctgctg aaagaacagg cgattatcga tatggccaaa 720
 aagcacggcg tcgaaccggc gcaactgatt atcagctggt cgattcagcg cggttatgtg 780
 gtattggcca agtcctgtaa tccggagcgt atcgtgtcga actttaagat tttaaccctg 840
 ccagaggatg atttcaaac catctctaac ctgagcaaaag tgcacggtag caaacgtgtc 900
 gttgacatga aatggggctc atttccgatt tttaataa 939

<210> 153

<211> 312

<212> PRT

<213> Artificial Sequence

<220>

<223> YPR ADH from *Saccharomyces cerevisiae*

<400> 153

Met Pro Ala Thr Leu Lys Asn Ser Ser Ala Thr Leu Lys Leu Asn Thr
 1 5 10 15
 Gly Ala Ser Ile Pro Val Leu Gly Phe Gly Thr Trp Arg Ser Val Asp
 20 25 30
 Asn Asn Gly Tyr His Ser Val Ile Ala Ala Leu Lys Ala Gly Tyr Arg
 35 40 45
 His Ile Asp Ala Ala Ala Ile Tyr Leu Asn Glu Glu Glu Val Gly Arg
 50 55 60
 Ala Ile Lys Asp Ser Gly Val Pro Arg Glu Glu Ile Phe Ile Thr Thr
 65 70 75 80
 Lys Leu Trp Gly Thr Glu Gln Arg Asp Pro Glu Ala Ala Leu Asn Lys

85 90 95
 Ser Leu Lys Arg Leu Gly Leu Asp Tyr Val Asp Leu Tyr Leu Met His
 100 105 110
 Trp Pro Val Pro Leu Lys Thr Asp Arg Val Thr Asp Gly Asn Val Leu
 115 120 125
 Cys Ile Pro Thr Leu Glu Asp Gly Thr Val Asp Ile Asp Thr Lys Glu
 130 135 140
 Trp Asn Phe Ile Lys Thr Trp Glu Leu Met Gln Glu Leu Pro Lys Thr
 145 150 155 160
 Gly Lys Thr Lys Ala Val Gly Val Ser Asn Phe Ser Ile Asn Asn Ile
 165 170 175
 Lys Glu Leu Leu Glu Ser Pro Asn Asn Lys Val Val Pro Ala Thr Asn
 180 185 190
 Gln Ile Glu Ile His Pro Leu Leu Pro Gln Asp Glu Leu Ile Ala Phe
 195 200 205
 Cys Lys Glu Lys Gly Ile Val Val Glu Ala Tyr Ser Pro Phe Gly Ser
 210 215 220
 Ala Asn Ala Pro Leu Leu Lys Glu Gln Ala Ile Ile Asp Met Ala Lys
 225 230 235 240
 Lys His Gly Val Glu Pro Ala Gln Leu Ile Ile Ser Trp Ser Ile Gln
 245 250 255
 Arg Gly Tyr Val Val Leu Ala Lys Ser Val Asn Pro Glu Arg Ile Val
 260 265 270
 Ser Asn Phe Lys Ile Phe Thr Leu Pro Glu Asp Asp Phe Lys Thr Ile
 275 280 285
 Ser Asn Leu Ser Lys Val His Gly Thr Lys Arg Val Val Asp Met Lys
 290 295 300
 Trp Gly Ser Phe Pro Ile Phe Gln
 305 310

<210> 154

<211> 1029

<212> DNA

<213> Artificial Sequence

<220>

<223> GRE ADH from *Saccharomyces cerevisiae*

<400> 154

atgtctgtgt tcgtgtcagg cgcgaatggf ttattgctc agcacatcgt agatctgctg 60
 ctgaaagaag attacaaagt aattggittcc gcacgtttctc aggaaaaaagc tgaataatttg 120
 accgaagccf tcgtaacaa cccgaaattt agcatggaag tgggtcctga tattagcaaa 180
 ctggatgcct tcgatcatgt attccagaaa catggtaaag atattaaaaat cgtcctgcat 240
 accgcgtccc cgttttgtt ccatattacc gattccgaac gtgacttact gatcccgcg 300
 gtaaacgggt tcaagggtat ttgcacagt attaagaaat atgccgcaga cagcgtggaa 360
 cgcgtggttc tgacttcac ttacgccggt gtatttgata tggcgaagga aaacgataag 420
 agcccgacct tcaacgaaga atcctggaat ccggcgacct gggaatcgtg tcagagtgat 480
 cgggtgaacg cttattgagg ttctaaaaaa ttccagagaa aagcagcgtg ggaattcttg 540
 gaagaaaaac gtgatagcgt gaaatttgag ctgacagcgg tcaaccaggt ttacgttttt 600
 ggtccgcaga tttcgataa agatgttaa aaacacttga acaccagctg cgaactggtg 660
 aactcttga tgactcag cctgaagat aaaattccgg aactgtttgg cggttacatc 720
 gacgtccgtg acgttcgaa agctcatctg gttgcatttc agaaacgtga aacaatcgtg 780
 cagcgccgtg tcgtgtcggg ggcaacttc acgatgcagg acgttcgga cattctgaac 840
 gaagacttcc cagtactgaa gggcaataac ccggtcggca agcctggcag cggcgccacc 900
 cataatactc tgggcccac cctggacaat aaaaaaagca aaaaattgct gggctcaaa 960
 ttccgtaatc tgaagagac tatgacgat actgcgtccc agatcctgaa attcgaaggt 1020
 cgcatttaa 1029

<210> 155

<211> 342

<212> PRT

<213> Artificial Sequence

<220>

<223> GRE ADH from *Saccharomyces cerevisiae*

<400> 155

Met Ser Val Phe Val Ser Gly Ala Asn Gly Phe Ile Ala Gln His Ile
 1 5 10 15
 Val Asp Leu Leu Leu Lys Glu Asp Tyr Lys Val Ile Gly Ser Ala Arg
 20 25 30
 Ser Gln Glu Lys Ala Glu Asn Leu Thr Glu Ala Phe Gly Asn Asn Pro
 35 40 45

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Lys Phe Ser Met Glu Val Val Pro Asp Ile Ser Lys Leu Asp Ala Phe
 50 55 60
 Asp His Val Phe Gln Lys His Gly Lys Asp Ile Lys Ile Val Leu His
 65 70 75 80
 Thr Ala Ser Pro Phe Cys Phe Asp Ile Thr Asp Ser Glu Arg Asp Leu
 85 90 95
 Leu Ile Pro Ala Val Asn Gly Val Lys Gly Ile Leu His Ser Ile Lys
 100 105 110
 Lys Tyr Ala Ala Asp Ser Val Glu Arg Val Val Leu Thr Ser Ser Tyr
 115 120 125
 Ala Ala Val Phe Asp Met Ala Lys Glu Asn Asp Lys Ser Leu Thr Phe
 130 135 140
 Asn Glu Glu Ser Trp Asn Pro Ala Thr Trp Glu Ser Cys Gln Ser Asp
 145 150 155 160
 Pro Val Asn Ala Tyr Cys Gly Ser Lys Lys Phe Ala Glu Lys Ala Ala
 165 170 175
 Trp Glu Phe Leu Glu Glu Asn Arg Asp Ser Val Lys Phe Glu Leu Thr
 180 185 190
 Ala Val Asn Pro Val Tyr Val Phe Gly Pro Gln Met Phe Asp Lys Asp
 195 200 205
 Val Lys Lys His Leu Asn Thr Ser Cys Glu Leu Val Asn Ser Leu Met
 210 215 220
 His Leu Ser Pro Glu Asp Lys Ile Pro Glu Leu Phe Gly Gly Tyr Ile
 225 230 235 240
 Asp Val Arg Asp Val Ala Lys Ala His Leu Val Ala Phe Gln Lys Arg
 245 250 255
 Glu Thr Ile Gly Gln Arg Leu Ile Val Ser Glu Ala Arg Phe Thr Met
 260 265 270
 Gln Asp Val Leu Asp Ile Leu Asn Glu Asp Phe Pro Val Leu Lys Gly
 275 280 285
 Asn Ile Pro Val Gly Lys Pro Gly Ser Gly Ala Thr His Asn Thr Leu
 290 295 300
 Gly Ala Thr Leu Asp Asn Lys Lys Ser Lys Lys Leu Leu Gly Phe Lys
 305 310 315 320
 Phe Arg Asn Leu Lys Glu Thr Ile Asp Asp Thr Ala Ser Gln Ile Leu
 325 330 335
 Lys Phe Glu Gly Arg Ile
 340