

BIOTECHNOLOGICAL PRODUCTION OF ω -FUNCTIONALISED CARBOXYLIC ACIDS AND ESTERS THEREOF

5 FIELD OF THE INVENTION

The present invention relates to a biotechnological method for producing ω -functionalized carboxylic acid esters. In particular, the method may use alkane as a starting material and a genetically modified cell to convert the alkane to the corresponding ω -functionalized carboxylic acid ester.

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BACKGROUND OF THE INVENTION

ω -functionalized carboxylic acids and the corresponding esters including ω -aminocarboxylic acids and their corresponding lactames, such as ω -aminolauric acid, ω -aminoundecanoic acid, laulactame and the like, are important monomers for production of high-performance polyamides. Some of the existing chemical technology that has been used to produce these monomers from petrochemical or renewable feedstocks include:

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- i) The production of ω -aminolauric acid from lauric acid methyl ester, a biodiesel fraction prepared from palm kernel or coconut oil
- 20 ii) The production of ω -aminoundecanoic acid from ricinoleic acid, prepared from castor oil
- iii) The production of laulactame from butadiene

While all these three methods of production remain competitive, their competitiveness at a given location and point in time is dependent on a number of factors, including the costs of raw materials and energy costs to run the methods.

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There are several biotechnological means of producing ω -functionalized carboxylic acids and/or esters thereof that are known in the art. For example, genetically modified cells that are capable of producing ω -functionalized carboxylic acids from carboxylic acid used as a substrate has been previously described at least in WO2009077461 and EP2322598. A very similar procedure is described in WO2011008232 using *Candida* cells where the β -oxidation pathway is blocked in the cells, and ω -functionalized carboxylic acids were formed from fatty acid used as a substrate. These methods have the disadvantage of using fatty acids as the starting material. This is because, the fatty acids and derivatives thereof used are mainly obtained exclusively from plant and animal oils or fats. Animal fats as raw materials still meet little client acceptance and plant oils which contain short- and middle-length fatty acids are either difficult to obtain or are produced only in tropical regions (result of destruction of rainforest). Further, particular plant and animal oil or fat raw materials have specific, but defined fatty acid profiles resulting in coupled production.

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WO2013024114 discloses a method of producing ω -functionalized carboxylic acids and/or esters thereof from simple carbon sources such as (glucose, saccharose, arabinose, xylose, lactose, fructose, maltose, molasses, starch, cellulose and hemicellulose, but also glycerin or very simple organic molecules such as CO₂, CO or synthesis gas). These simple sugars especially glucose are usually more expensive to obtain. The method of producing ω -functionalized carboxylic acids and/or esters thereof from simple carbon sources may also be considered complicated as the cells used in this method have to be genetically modified to increase production of carboxylic acids from these simple carbon sources first. This thus increases the cost of production.

Therefore, there is a need in the art for a method to produce ω -functionalized carboxylic acids esters from another source of raw material that enables the production to be efficient and effective.

DESCRIPTION OF THE INVENTION

The present invention attempts to solve the problems above by providing at least one genetically modified microbial cell that is capable of producing at least one ω -functionalized carboxylic acid ester from at least one alkane. The ω -functionalized carboxylic acid ester may be selected from the group consisting of ω -hydroxy, ω -oxo, ω -carboxy and ω -amino carboxylic acid esters. The use of these genetically modified cells in a method to produce ω -functionalized carboxylic acid esters may add flexibility to the production of these compounds by enabling the use of a readily available alternative petrochemical raw material for its production. Also, the use of whole-cell biocatalysts capable of integrating the entire means of converting alkanes to fatty acid esters and their corresponding ω -functionalized derivatives within them, makes the process of conversion simpler as only a small number of process steps are involved in the conversion. The reliance of fatty acids and simple carbon sources as the carbon substrate is also eliminated.

According to a first aspect of the present invention, there is provided a microbial cell for producing at least one ω -functionalized carboxylic acid ester from at least one alkane, wherein the cell comprises a genetic modification to increase the expression relative to the wild type cell of

- (i) Enzyme E₁ capable of converting the alkane to the corresponding 1-alkanol;
- (ii) Enzyme E₂ capable of converting the 1-alkanol of (i) to the corresponding 1-alkanal;
- (iii) Enzyme E₃ capable of converting the 1-alkanal of (ii) to the corresponding alkanoic acid;
- (iv) Enzyme E₄ capable of converting the alkanoic acid of (iii) to the corresponding alkanoic acid ester; and
- (v) Enzyme E₅ capable of converting the alkanoic acid ester of (iv) to the corresponding ω -hydroxy-alkanoic acid ester.

The microbial cell according to any aspect of the present invention refers to a cell that has no previous/former modification to increase the formation of carboxylic acid or carboxylate ester from at least one simple carbon source. The term "simple carbon source" is understood to mean carbon sources wherein in the carbon skeleton at least one C-C bond has been broken. In particular, the simple carbon source may be at least one carbohydrate such as for example glucose, saccharose, arabinose, xylose, lactose, fructose, maltose, molasses, starch, cellulose and hemicellulose, but carbon sources may also include glycerin or very simple organic molecules such as CO₂, CO or synthesis gas. More in particular, the microbial cell according to any aspect of the present invention may not be genetically modified to increase the expression relative to the wild type cell of at least one of the following enzymes:

- E₂₀ Acyl-ACP thioesterase, of EC 3.1.2.14 or EC 3.1.2.22,
- E₂₁ Acyl-CoA thioesterase, of EC 3.1.2.2, EC 3.1.2.18, EC 3.1.2.19, EC 3.1.2.20 or EC 3.1.2.22,
- E₂₂ Acyl-CoA:ACP transacylase,
- E₂₃ Polyketide synthase, which catalyses a reaction which is involved in the synthesis of carboxylic acids and carboxylate esters, and
- E₂₄ Hexanoic acid synthase.

In one example, the cell according to any aspect of the present invention may comprise a further genetic modification to increase the expression relative to the wild type cell of

- (vi) Enzyme E₆ capable of converting the ω -hydroxy-alkanoic acid ester of (v) to the corresponding ω -oxo alkanoic acid ester; and
- (vii) Enzyme E₇ capable of converting the ω -oxo alkanoic acid ester of (vi) to the corresponding ω -amino alkanoic acid ester.

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In another example, the cell according to any aspect of the present invention may comprise a further genetic modification to increase the expression relative to the wild type cell of

- (vi) Enzyme E₆ capable of converting ω -hydroxy-alkanoic acid ester of (v) to the corresponding ω -oxo alkanoic acid ester;
- (vii) Enzyme E₁₃ capable of converting the ω -oxo alkanoic acid ester of (vi) to the corresponding ω -carboxy alkanoic acid ester, and
- (viii) Enzyme E₁₄ capable of converting the ω -carboxy alkanoic acid ester of (vi) to the corresponding ω -carboxy alkanoic acid diester.

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The cells according to any aspect of the present invention may be used to produce ω -functionalized carboxylic acid ester from all alkanes with high space-time yield, high carbon yield and high concentration in the culture supernatant. As a result of these advantages, an efficient workup is facilitated.

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The phrase "wild type" as used herein in conjunction with a cell or microorganism may denote a cell with a genome make-up that is in a form as seen naturally in the wild. The term may be applicable for both the whole cell and for individual genes. The term 'wild type' may thus also include cells which have been genetically modified in other aspects (i.e. with regard to one or more genes) but not in relation to the genes of interest. The term "wild type" therefore does not include such cells where the gene sequences of the specific genes of interest have been altered at least partially by man using recombinant methods. A wild type cell according to any aspect of the present invention thus refers to a cell that has no genetic mutation with respect to the whole genome and/or a particular gene. Therefore, in one example, a wild type cell with respect to enzyme E₁ may refer to a cell that has the natural/ non-altered expression of the enzyme E₁ in the cell. The wild type cell with respect to enzyme E₂, E₃, E₄, E₅, E₆, E₇, E₈, E₉, E₁₀, E₁₁, E₁₂, E₁₃, E₁₄, E₁₅, etc. may be interpreted the same way and may refer to a cell that has the natural/ non-altered expression of the enzyme E₂, E₃, E₄, E₅, E₆, E₇, E₈, E₉, E₁₀, E₁₁, E₁₂, E₁₃, E₁₄, E₁₅, etc. respectively in the cell.

Any of the enzymes used according to any aspect of the present invention, may be an isolated enzyme. In particular, the enzymes used according to any aspect of the present invention may be used in an active state and in the presence of all cofactors, substrates, auxiliary and/or activating polypeptides or factors essential for its activity. The term "isolated", as used herein, means that the enzyme of interest is enriched compared to the cell in which it occurs naturally. The enzyme may be enriched by SDS polyacrylamide electrophoresis and/or activity assays. For example, the enzyme of interest may constitute more than 5, 10, 20, 50, 75, 80, 85, 90, 95 or 99 percent of all the polypeptides present in the preparation as judged by visual inspection of a polyacrylamide gel following staining with Coomassie blue dye.

The enzyme used according to any aspect of the present invention may be recombinant. The term "recombinant" as used herein, refers to a molecule or is encoded by such a molecule, particularly a polypeptide or nucleic acid that, as such, does not occur naturally but is the result of genetic engineering or refers to a cell that comprises a recombinant molecule. For example, a nucleic acid molecule is recombinant if it comprises a promoter functionally linked to a sequence encoding a catalytically active polypeptide and the promoter has been engineered such that the catalytically active polypeptide is overexpressed relative to the level of the polypeptide in the corresponding wild type cell that comprises the original unaltered nucleic acid molecule.

A skilled person would be able to use any method known in the art to genetically modify a cell or microorganism. According to any aspect of the present invention, the genetically modified cell may be genetically modified so that in a defined time interval, within 2 hours, in particular within 8 hours or 24 hours, it forms at least once or twice, especially at least 10 times, at least 100 times, at least 1000 times or at least 10000 times more ω -functionalized carboxylic acid ester than the wild-type cell. The increase in product formation can be determined for example by cultivating the cell according to any aspect of the present invention and the wild-type cell each separately under the

same conditions (same cell density, same nutrient medium, same culture conditions) for a specified time interval in a suitable nutrient medium and then determining the amount of target product (ω -functionalized carboxylic acid ester) in the nutrient medium.

- 5 The genetically modified cell or microorganism may be genetically different from the wild type cell or microorganism. The genetic difference between the genetically modified microorganism according to any aspect of the present invention and the wild type microorganism may be in the presence of a complete gene, amino acid, nucleotide etc. in the genetically modified microorganism that may be absent in the wild type microorganism. In one example, the genetically modified
- 10 microorganism according to any aspect of the present invention may comprise enzymes that enable the microorganism to produce more 1-alkanols, 1-alkanals, alkanolic acids, alkanolic acid esters, omega-hydroxy alkanolic acid esters etc. compared to the wild type cells. The wild type microorganism relative to the genetically modified microorganism of the present invention may have none or no detectable activity of the enzymes that enable the genetically modified
- 15 microorganism to produce 1-alkanols, 1-alkanals, alkanolic acids, alkanolic acid esters, omega-hydroxy alkanolic acid esters, etc. As used herein, the term 'genetically modified microorganism' may be used interchangeably with the term 'genetically modified cell'. The genetic modification according to any aspect of the present invention is carried out on the cell of the microorganism.
- 20 The cells according to any aspect of the present invention are genetically transformed according to any method known in the art. In particular, the cells may be produced according to the method disclosed in WO2013024114.

25 The phrase 'the genetically modified cell has an increased activity, in comparison with its wild type, in enzymes' as used herein refers to the activity of the respective enzyme that is increased by a factor of at least 2, in particular of at least 10, more in particular of at least 100, yet more in particular of at least 1000 and even more in particular of at least 10000.

30 The phrase "increased activity of an enzyme", as used herein is to be understood as increased intracellular activity. Basically, an increase in enzymatic activity can be achieved by increasing the copy number of the gene sequence or gene sequences that code for the enzyme, using a strong promoter or employing a gene or allele that codes for a corresponding enzyme with increased activity, altering the codon utilization of the gene, increasing the half-life of the mRNA or of the enzyme in various ways, modifying the regulation of the expression of the gene and optionally by

35 combining these measures. Genetically modified cells used according to any aspect of the present invention are for example produced by transformation, transduction, conjugation or a combination of these methods with a vector that contains the desired gene, an allele of this gene or parts thereof and a vector that makes expression of the gene possible. Heterologous expression is in particular achieved by integration of the gene or of the alleles in the chromosome of the cell or an

40 extrachromosomally replicating vector

In the same context, the phrase "decreased activity of an enzyme Ex" used with reference to any aspect of the present invention may be understood as meaning an activity decreased by a factor of at least 0.5, particularly of at least 0.1, more particularly of at least 0.01, even more particularly of at least 0.001 and most particularly of at least 0.0001. The phrase "decreased activity" also comprises no detectable activity ("activity of zero"). The decrease in the activity of a certain enzyme can be effected, for example, by selective mutation or by other measures known to the person skilled in the art for decreasing the activity of a certain enzyme. In particular, the person skilled in the art finds instructions for the modification and decrease of protein expression and concomitant lowering of enzyme activity by means of interrupting specific genes, for example at least in Dubeau *et al.* 2009. Singh & Röhm. 2008., Lee *et al.*, 2009 and the like. The decrease in the enzymatic activity in a cell according to any aspect of the present invention may be achieved by modification of a gene comprising one of the nucleic acid sequences, wherein the modification is selected from the group comprising, consisting of, insertion of foreign DNA in the gene, deletion of at least parts of the gene, point mutations in the gene sequence, RNA interference (siRNA), *antisense* RNA or modification (insertion, deletion or point mutations) of regulatory sequences, such as, for example, promoters and terminators or of ribosome binding sites, which flank the gene.

Foreign DNA is to be understood in this connection as meaning any DNA sequence which is "foreign" to the gene (and not to the organism), i.e. endogenous DNA sequences can also function in this connection as "foreign DNA". In this connection, it is particularly preferred that the gene is interrupted by insertion of a selection marker gene, thus the foreign DNA is a selection marker gene, wherein preferably the insertion was effected by homologous recombination in the gene locus.

The expression of the enzymes and genes mentioned above and all mentioned below is determinable by means of 1- and 2-dimensional protein gel separation followed by optical identification of the protein concentration in the gel with appropriate evaluation software. If the increasing of an enzyme activity is based exclusively on increasing the expression of the corresponding gene, then the quantification of the increasing of the enzyme activity can be simply determined by a comparison of the 1- or 2-dimensional protein separations between wild type and genetically modified cell. A common method for the preparation of the protein gels with bacteria and for identification of the proteins is the procedure described by Hermann *et al.* (Electrophoresis, 22: 1712-23 (2001)). The protein concentration can also be analysed by Western blot hybridization with an antibody specific for the protein to be determined (Sambrook *et al.*, Molecular Cloning: a laboratory manual, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. USA, 1989) followed by optical evaluation with appropriate software for concentration determination (Lohaus and Meyer (1989) Biospektrum, 5: 32-39; Lottspeich (1999), Angewandte Chemie 111: 2630-2647). This method is also always an option when possible products of the reaction to be catalysed by the enzyme activity to be determined may be rapidly metabolized in the

microorganism or else the activity in the wild type is itself too low for it to be possible adequately to determine the enzyme activity to be determined on the basis of the production formation.

In particular,

- 5 - the Enzyme E₁ may be selected from the group consisting of P450 alkane hydroxylases (E_a) and AlkB alkane hydroxylases (E_b) of EC 1.14.15.3;
 - the Enzyme E₂ may be selected from the group consisting of P450 alkane hydroxylases (E_a), AlkB alkane hydroxylases (E_b) of EC 1.14.15.3, alcohol oxidases (E_c) of EC 1.1.3.20 and alcohol dehydrogenases (E_d);
 - 10 - the Enzyme E₃ is selected from the group consisting of P450 alkane hydroxylases (E_a) of EC 1.14.15.3-, AlkB alkane hydroxylases (E_b) of EC 1.14.15.3, aldehyde dehydrogenases (E_e), bifunctional alcohol oxidases (E_c) of EC 1.1.3.20, bifunctional AlkJ alcohol dehydrogenases (E_{di}) and bifunctional alcohol dehydrogenases (E_{dii}) of EC 1.1.1.1 or EC 1.1.1.2, wherein E_c, E_{di}, and E_{dii} are capable of oxidizing an 1-alkanol via an 1-alkanal directly to the corresponding alkanolic acid;
 - 15 - the Enzyme E₄ may be selected from the group consisting of wax-ester synthases (E_f) and alcohol O-acyl transferases (E_g);
 - the Enzyme E₅ may be selected from the group consisting of P450 alkane hydroxylases (E_a) and AlkB alkane hydroxylases (E_b) of EC 1.14.15.3;
 - 20 - the Enzyme E₆ may be selected from the group consisting of P450 alkane hydroxylases (E_a), AlkB alkane hydroxylases (E_b) of EC 1.14.15.3, alcohol oxidases (E_c) and alcohol dehydrogenases (E_d);
 - the Enzyme E₇ may be an ω -transaminase (E_h).
- 25 In one example, enzymes E₁, E₂, E₃ and E₅ may each be different enzymes that may be capable of carrying out their activity. For example, E₁ may be AlkB alkane hydroxylases (E_b), E₂ may be alcohol oxidase (E_c), E₃ may be bifunctional AlkJ alcohol dehydrogenase, and E₅ may be AlkB alkane hydroxylase (E_b). In another example, E₁ may be P450 alkane hydroxylase (E_a), E₂ may be alcohol dehydrogenase (E_c), E₃ may be aldehyde dehydrogenase (E_e), and E₅ may P450 alkane
- 30 hydroxylase. In particular, any combination of enzymes of E₁, E₂, E₃ and E₅ may be used to carry out their specific functions. In a further example, E₁, E₂, E₃ and E₅ may be the same enzyme. In this example, E₁, E₂, E₃ and E₅ may be selected from the group consisting of P450 alkane hydroxylases (E_a) and AlkB alkane hydroxylases (E_b). In one example, E₁, E₂, E₃ and E₅ may be P450 alkane hydroxylases (E_a). In this example, the cell according to any aspect of the present invention
- 35 comprises an increased expression relative to the wild type cell of P450 alkane hydroxylases (E_a) that satisfies the function of enzymes E₁, E₂, E₃ and E₅. In another example, E₁, E₂, E₃ and E₅ may be AlkB alkane hydroxylase (E_b). In this example, the cell according to any aspect of the present invention comprises an increased expression relative to the wild type cell of AlkB alkane hydroxylase (E_b) that satisfies the function of enzymes E₁, E₂, E₃ and E₅.

The enzymes E_a to E_n may comprise a polypeptide sequence wherein up to 60%, preferably up to 25%, particularly up to 15%, in particular up to 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1% of the amino acid residues are modified compared to the below reference sequences (accession numbers by deletion, insertion, substitution or a combination thereof and which still possess at least 50%, preferably 65%, particularly preferably 80%, in particular more than 90% of the activity of the protein with the corresponding, below reference sequence, wherein 100% activity of the reference protein is understood to mean the increasing of the activity of the cells used as a biocatalyst, i.e. the quantity of substance converted per unit time based on the cell quantity used (units per gram cell dry weight [U/g CDW]) in comparison to the activity of the biocatalyst in the absence of the reference protein.

Modifications of amino acid residues of a given polypeptide sequence which lead to no significant modifications of the properties and function of the given polypeptide are known to those skilled in the art. Thus for example many amino acids can often be exchanged for one another without problems; examples of such suitable amino acid substitutions are: Ala by Ser; Arg by Lys; Asn by Gln or His; Asp by Glu; Cys by Ser; Gln by Asn; Glu by Asp; Gly by Pro; His by Asn or Gln; Ile by Leu or Val; Leu by Met or Val; Lys by Arg or Gln or Glu; Met by Leu or Ile; Phe by Met or Leu or Tyr; Ser by Thr; Thr by Ser; Trp by Tyr; Tyr by Trp or Phe; Val by Ile or Leu. It is also known that modifications, particularly at the N- or C-terminus of a polypeptide in the form of for example amino acid insertions or deletions, often exert no significant influence on the function of the polypeptide.

The accession numbers stated in connection with the present invention mentioned throughout this specification correspond to the NCBI ProteinBank database entries with the date 26.07.2011; as a rule, the version number of the entry is identified here by "numerals" such as for example ".1".

All stated percentages (%) are, unless otherwise stated, mass percent.

According to any aspect of the present invention, the microbial cell may be selected from the species of bacteria from the group consisting of, *Abiotrophia*, *Acaryochloris*, *Accumulibacter*, *Acetivibrio*, *Acetobacter*, *Acetohalobium*, *Acetonema*, *Achromobacter*, *Acidaminococcus*, *Acidimicrobium*, *Acidiphilium*, *Acidithiobacillus*, *Acidobacterium*, *Acidotherrmus*, *Acidovorax*, *Acinetobacter*, *Actinobacillus*, *Actinomyces*, *Actinosynnema*, *Aerococcus*, *Aeromicrobium*, *Aeromonas*, *Afipia*, *Aggregatibacter*, *Agrobacterium*, *Ahrensia*, *Akkermansia*, *Alcanivorax*, *Alicyclophilus*, *Alicyclobacillus*, *Aliivibrio*, *Alkalilimnicola*, *Alkaliphilus*, *Allochromatium*, *Alteromonadales*, *Alteromonas*, *Aminobacterium*, *Aminomonas*, *Ammonifex*, *Amycolatopsis*, *Amycolalicoccus*, *Anabaena*, *Anaerobaculum*, *Anaerococcus*, *Anaerofustis*, *Anaerolinea*, *Anaeromyxobacter*, *Anaerostipes*, *Anaerotruncus*, *Anaplasma*, *Anoxybacillus*, *Aquifex*, *Arcanobacterium*, *Arcobacter*, *Aromatoleum*, *Arthrobacter*, *Arthrospira*, *Asticcacaulis*, *Atopobium*, *Aurantimonas*, *Azoarcus*, *Azorhizobium*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Bartonella*, *Basfia*, *Baumannia*, *Bdellovibrio*, *Beggiatoa*, *Beijerinckia*, *Bermanella*, *Beutenbergia*, *Bifidobacterium*,

- Bilophila*, *Blastopirellula*, *Blautia*, *Blochmannia*, *Bordetella*, *Borrelia*, *Brachybacterium*, *Brachyspira*, *Bradyrhizobium*, *Brevibacillus*, *Brevibacterium*, *Brevundimonas*, *Brucella*, *Buchnera*, *Bulleidia*, *Burkholderia*, *Butyrivibrio*, *Caldalkalibacillus*, *Caldanaerobacter*, *Caldicellulosiruptor*, *Calditerrivibrio*, *Caminibacter*, *Campylobacter*, *Carboxydibrachium*, *Carboxydotherrmus*,
- 5 *Cardiobacterium*, *Carnobacterium*, *Carsonella*, *Catenibacterium*, *Catenulispora*, *Catonella*, *Caulobacter*, *Cellulomonas*, *Cellvibrio*, *Centipeda*, *Chelativorans*, *Chloroflexus*, *Chromobacterium*, *Chromohalobacter*, *Chthoniobacter*, *Citricella*, *Citrobacter*, *Citromicrobium*, *Clavibacter*, *Cloacamonas*, *Clostridium*, *Collinsella*, *Colwellia*, *Comamonas*, *Conexibacter*, *Congregibacter*, *Coprobacillus*, *Coprococcus*, *Coprothermobacter*, *Coralimargarita*, *Coriobacterium*, *corrodens*,
- 10 *Corynebacterium*, *Coxiella*, *Crocospaera*, *Cronobacter*, *Cryptobacterium*, *Cupriavidus*, *Cyanobium*, *Cyanothece*, *Cylindrospermopsis*, *Dechloromonas*, *Deferribacter*, *Dehalococcoides*, *Dehalogenimonas*, *Deinococcus*, *Delftia*, *Denitrovibrio*, *Dermacoccus*, *Desmospora*, *Desulfarculus*, *Desulphateibacillum*, *Desulfitobacterium*, *Desulfobacca*, *Desulfobacterium*, *Desulfobulbus*, *Desulfococcus*, *Desulfohalobium*, *Desulfomicrobium*, *Desulfonatronospira*, *Desulforudis*,
- 15 *Desulfotalea*, *Desulfotomaculum*, *Desulfovibrio*, *Desulfurispirillum*, *Desulfurobacterium*, *Desulfuromonas*, *Dethiobacter*, *Dethiosulfovibrio*, *Dialister*, *Dichelobacter*, *Dickeya*, *Dictyoglomus*, *Dietzia*, *Dinoroseobacter*, *Dorea*, *Edwardsiella*, *Ehrlichia*, *Eikenella*, *Elusimicrobium*, *Endoriftia*, *Enhydrobacter*, *Enterobacter*, *Enterococcus*, *Epulopiscium*, *Erwinia*, *Erysipelothrix*, *Erythrobacter*, *Escherichia*, *Ethanoligenens*, *Eubacterium*, *Eubacterium*, *Exiguobacterium*, *Faecalibacterium*,
- 20 *Ferrimonas*, *Fervidobacterium*, *Fibrobacter*, *Fingoldia*, *Flexistipes*, *Francisella*, *Frankia*, *Fructobacillus*, *Fulvimarina*, *Fusobacterium*, *Gallibacterium*, *Gallionella*, *Gardnerella*, *Gemella*, *Gemmata*, *Gemmatimonas*, *Geobacillus*, *Geobacter*, *Geodermatophilus*, *Glaciecola*, *Gloeobacter*, *Glossina*, *Gluconacetobacter*, *Gordonia*, *Granulibacter*, *Granulicatella*, *Grimontia*, *Haemophilus*, *Hahella*, *Halanaerobiumns*, *Haliangium*, *Halomonas*, *Halorhodospira*, *Halothermothrix*,
- 25 *Halothiobacillus*, *Hamiltonella*, *Helicobacter*, *Heliobacterium*, *Herbaspirillum*, *Herminiimonas*, *Herpetosiphon*, *Hippea*, *Hirschia*, *Histophilus*, *Hodgkinia*, *Hoeflea*, *Holdemania*, *Hydrogenivirga*, *Hydrogenobaculum*, *Hylemonella*, *Hyphomicrobium*, *Hyphomonas*, *Idiomarina*, *Ilyobacter*, *Intrasporangium*, *Isoptericola*, *Isosphaera*, *Janibacter*, *Janthinobacterium*, *Jonesia*, *Jonquetella*, *Kangiella*, *Ketogulonicigenium*, *Kineococcus*, *Kingella*, *Klebsiella*, *Kocuria*, *Koribacter*, *Kosmotoga*,
- 30 *Kribbella*, *Ktedonobacter*, *Kytococcus*, *Labrenzia*, *Lactobacillus*, *Lactococcus*, *Laribacter*, *Lautropia*, *Lawsonia*, *Legionella*, *Leifsonia*, *Lentisphaera*, *Leptolyngbya*, *Leptospira*, *Leptothrix*, *Leptotrichia*, *Leuconostoc*, *Liberibacter*, *Limnobacter*, *Listeria*, *Loktanella*, *Lutiella*, *Lyngbya*, *Lysinibacillus*, *Macrococcus*, *Magnetococcus*, *Magnetospirillum*, *Mahella*, *Mannheimia*, *Maricaulis*, *Marinithermus*, *Marinobacter*, *Marinomonas*, *Mariprofundus*, *Maritimibacter*, *Marvinbryantia*,
- 35 *Megasphaera*, *Meiothermus*, *Melissococcus*, *Mesorhizobium*, *Methylacidiphilum*, *Methylibium*, *Methylobacillus*, *Methylobacter*, *Methylobacterium*, *Methylococcus*, *Methylocystis*, *Methylomicrobium*, *Methylophaga*, *Methylophilales*, *Methylosinus*, *Methyloversatilis*, *Methylovorus*, *Microbacterium*, *Micrococcus*, *Microcoleus*, *Microcystis*, *Microlunatus*, *Micromonospora*, *Mitsuokella*, *Mobiluncus*, *Moorella*, *Moraxella*, *Moritella*, *Mycobacterium*, *Myxococcus*,
- 40 *Nakamurella*, *Natranaerobius*, *Neisseria*, *Neorickettsia*, *Neptuniibacter*, *Nitratifractor*, *Nitratiruptor*,

- Nitrobacter*, *Nitrococcus*, *Nitrosomonas*, *Nitrosospira*, *Nitrospira*, *Nocardia*, *Nocardioides*,
Nocardiopsis, *Nodularia*, *Nostoc*, *Novosphingobium*, *Oceanibulbus*, *Oceanicaulis*, *Oceanicola*,
Oceanithermus, *Oceanobacillus*, *Ochrobactrum*, *Octadecabacter*, *Odyssella*, *Oligotropha*,
Olsenella, *Opitutus*, *Oribacterium*, *Orientia*, *Ornithinibacillus*, *Oscillatoria*, *Oscillochloris*,
5 *Oxalobacter*, *Paenibacillus*, *Pantoea*, *Paracoccus*, *Parascardovia*, *Parasutterella*, *Parvibaculum*,
Parvimonas, *Parvularcula*, *Pasteurella*, *Pasteuria*, *Pectobacterium*, *Pediococcus*, *Pedosphaera*,
Pelagibaca, *Pelagibacter*, *Pelobacter*, *Pelotomaculum*, *Peptoniphilus*, *Peptostreptococcus*,
Persephonella, *Petrogla*, *Phaeobacter*, *Phascolarctobacterium*, *Phenylobacterium*,
Photobacterium, *Pirellula*, *Planctomyces*, *Planococcus*, *Plesiocystis*, *Polaromonas*, *Polaromonas*,
10 *Polymorphum*, *Polynucleobacter*, *Poribacteria*, *Prochlorococcus*, *Propionibacterium*, *Proteus*,
Providencia, *Pseudoalteromonas*, *Pseudoflavonifractor*, *Pseudomonas*, *Pseudonocardia*,
Pseudoramibacter, *Pseudovibrio*, *Pseudoxanthomonas*, *Psychrobacter*, *Psychromonas*,
Puniceispirillum, *Pusillimonas*, *Pyramidobacter*, *Rahnella*, *Ralstonia*, *Raphidiopsis*, *Regiella*,
Reinekea, *Renibacterium*, *Rhizobium*, *Rhodobacter*, *Rhodococcus*, *Rhodoferax*, *Rhodomicrobium*,
15 *Rhodopirellula*, *Rhodopseudomonas*, *Rhodospirillum*, *Rickettsia*, *Rickettsiella*, *Riesia*, *Roseburia*,
Roseibium, *Roseiflexus*, *Roseobacter*, *Roseomonas*, *Roseovarius*, *Rothia*, *Rubrivivax*,
Rubrobacter, *Ruegeria*, *Ruminococcus*, *Ruthia*, *Saccharomonospora*, *Saccharophagus*,
Saccharopolyspora, *Sagittula*, *Salinispora*, *Salmonella*, *Sanguibacter*, *Scardovia*, *Sealdella*,
Segniliparus, *Selenomonas*, *Serratia*, *Shewanella*, *Shigella*, *Shuttleworthia*, *Sideroxydans*,
20 *Silicibacter*, *Simonsiella*, *Sinorhizobium*, *Slackia*, *Sodalis*, *Solibacter*, *Solobacterium*, *Sorangium*,
Sphaerobacter, *Sphingobium*, *Sphingomonas*, *Sphingopyxis*, *Spirochaeta*, *Sporosarcina*,
Stackebrandtia, *Staphylococcus*, *Starkeya*, *Stenotrophomonas*, *Stigmatella*, *Streptobacillus*,
Streptococcus, *Streptomyces*, *Streptosporangium*, *Subdoligranulum*, *subvibrioides*,
Succinatimonas, *Sulfitobacter*, *Sulfobacillus*, *Sulfuricurvum*, *Sulfurihydrogenibium*, *Sulfurimonas*,
25 *Sulfurospirillum*, *Sulfurovum*, *Sutterella*, *Symbiobacterium*, *Synechocystis*, *Syntrophobacter*,
Syntrophobotulus, *Syntrophomonas*, *Syntrophothermus*, *Syntrophus*, *taiwanensis*, *Taylorella*,
Teredinibacter, *Terriglobus*, *Thalassibium*, *Thauera*, *Thermaerobacter*, *Thermanaerovibrio*,
Thermincola, *Thermoanaerobacter*, *Thermoanaerobacterium*, *Thermobaculum*, *Thermobifida*,
Thermobispora, *Thermocrinis*, *Thermodesulphateator*, *Thermodesulfobacterium*,
30 *Thermodesulfobium*, *Thermodesulfobivibrio*, *Thermomicrobium*, *Thermomonospora*,
Thermosediminibacter, *Thermosinus*, *Thermosipho*, *Thermosynechococcus*, *Thermotoga*,
Thermovibrio, *Thermus*, *Thioalkalimicrobium*, *Thioalkalivibrio*, *Thiobacillus*, *Thiomicrospira*,
Thiomonas, *Tolomonas*, *Treponema*, *tribocorum*, *Trichodesmium*, *Tropheryma*, *Truepera*,
Tsukamurella, *Turicibacter*, *Variovorax*, *Veillonella*, *Verminephrobacter*, *Verrucomicrobium*,
35 *Verrucosispora*, *Vesicomysocius*, *Vibrio*, *Vibrionales*, *Victivallis*, *Weissella*, *Wigglesworthia*,
Wolbachia, *Wolinella*, *Xanthobacter*, *Xanthomonas*, *Xenorhabdus*, *Xylanimonas*, *Xylella*, *Yersinia*,
Zinderia and *Zymomonas*,

- In particular, the microbial cell may be from *E. coli*, *Pseudomonas* sp., *Pseudomonas fluorescens*,
40 *Pseudomonas putida*, *Pseudomonas stutzeri*, *Acinetobacter* sp., *Burkholderia* sp., *Burkholderia*

thailandensis, Cyanobakterien, *Klebsiella* sp., *Klebsiella oxytoca*, *Salmonella* sp., *Rhizobium* sp. and *Rhizobium meliloti*, *Bacillus* sp., *Bacillus subtilis*, *Clostridium* sp., *Corynebacterium* sp., *Corynebacterium glutamicum*, *Brevibacterium* sp., *Chlorella* sp. and *Nostoc* sp.. More in particular, the microbial cell may be from *E. coli*.

5

Alkanes are saturated hydrocarbons that have various applications depending on the number of carbon atoms and on the structure of the alkane (i.e. branched, linear, cyclic etc.). Alkanes (technically, always acyclic or open-chain compounds) have the general chemical formula C_nH_{2n+2} . An alkane used according to any aspect of the present invention may comprise at least 6 C atoms.

10

In particular, the alkane used according to any aspect of the present invention may comprise 6-22, 6-20, 6-18, 6-17, 6-16, 6-15, 6-14, 6-13, 6-12, 6-11, 6-10, 8-20, 8-19, 8-18, 8-16, 8-15, 8-12, 8-10 carbon atoms (inclusive). The alkanes may be selected from the group consisting of hexane, heptane, octane, nonane, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, and icosane. In particular, the alkane used may be decane, undecane or dodecane.

15

Enzyme E₁

Enzyme E₁ may be capable of converting at least one alkane to the corresponding 1-alkanol. In particular, E₁ may be at least one P450 alkane hydroxylase (E_a) of EC 1.14.15.1 or AlkB alkane hydroxylase (E_b) of EC 1.14.15.3. The P450 alkane hydroxylase (E_a) is a component of a reaction system comprising

20

- two enzyme components cytochrome P450 alkane hydroxylase and NAD(P)H cytochrome P450 oxidoreductase of EC 1.6.2.4 or
- three enzyme components cytochrome P450 alkane hydroxylase of the CYP153 type, ferredoxin NAD(P)⁺ reductases of EC 1.18.1.2 or EC 1.18.1.3 and ferredoxin.

25

The AlkB alkane hydroxylase (E_{1b}) is a component of a reaction system comprising

- AlkB alkane hydroxylases of EC 1.14.15.3 which is a component of a reaction system comprising three enzyme components AlkB alkane hydroxylase of EC 1.14.15.3, AlkT rubredoxin NAD(P)⁺ reductase of EC 1.18.1.1 or of EC 1.18.1.4 and rubredoxin AlkG.

30

In particular, E₁ may be an AlkB alkane hydroxylase (E_b) also known as an alkane monooxygenase. More in particular, E₁ may comprise sequence identity of at least 50% to the alkane monooxygenase from *Pseudomonas putida* GPo1 encoded by alkBGT. Even more in particular, E₁ may comprise sequence identity of at least 50% to the polypeptide YP_001185946.1. More in particular, E₁ may comprise a polypeptide with sequence identity of at least 50, 60, 65, 70, 75, 80, 85, 90, 91, 94, 95, 98 or 100% to a polypeptide YP_001185946.1.

35

Enzyme E_a

In particular, the enzyme E₁ may at least be one P450 alkane hydroxylases (E_a) selected from the group consisting of:

AAO73954.1, AAO73953.1, XP_002546279.1, AAA34353.2, P30607.1, XP_002421627.1,
 XP_718670.1, CAA39366.1, XP_001527524.1, AAO73955.1, AAO73956.1, XP_002546278.1,
 EEQ43157.1, XP_718669.1, AAA34354.1, P10615.3, XP_002421628.1, 226487, P16141.3,
 CAA39367.1, Q9Y757.2, XP_001485567.1, AAO73958.1, XP_001383506.2, XP_460111.2,
 5 AAO73959.1, Q12586.1, XP_460112.2, AAO73960.1, Q12589.1, AAO73961.1, XP_460110.2,
 EEQ43763.1, XP_710174.1, EDK41572.2, XP_001482650.1, CAA75058.1, XP_002548818.1,
 Q12588.1, XP_002422222.1, XP_001383636.2, XP_001525381.1, XP_002548823.1, P30610.1,
 AAO73952.1, XP_002548428.1, CAA36197.1, XP_002421126.1, AAA34320.1, P16496.3,
 P30608.1, P24458.1, XP_717999.1, XP_001383817.1, Q9Y758.1, XP_001482092.1,
 10 XP_001383710.2, P30609.1, AAB24479.1, XP_457792.1, XP_001524144.1, XP_457727.2,
 XP_001525578.1, XP_002616743.1, XP_002614836.1, XP_001525577.1, AAO73957.1,
 Q12585.1, XP_001386440.2, XP_002616857.1, XP_001483276.1, XP_500402.1, EDK39907.2,
 XP_500560.1, XP_001211376.1, XP_002560027.1, XP_504857.1, XP_500855.1, XP_504406.1,
 BAA31433.1, XP_500856.1, XP_501148.1, XP_746567.1, XP_001262425.1, XP_001274843.1,
 15 XP_002840588.1, XP_002377641.1, XP_001825995.1, XP_001400739.1, XP_718066.1,
 CAA35593.1, XP_664735.1, XP_002150795.1, XP_500097.1, XP_002483325.1, XP_504311.1,
 XP_500273.1, XP_002548817.1, EDP54484.1, XP_755288.1, XP_001260447.1, EFY97851.1,
 ACD75398.1, ADK36660.1, XP_001213081.1, XP_002377989.1, XP_001826299.1,
 XP_001554811.1, XP_501667.1, XP_002148942.1, ADK36662.1, XP_002565827.1, P30611.1,
 20 XP_001267871.1, XP_002372373.1, EFY84686.1, P43083.1, XP_001263094.1, XP_002148355.1,
 XP_002568429.1, XP_001817314.1, Q12587.1, XP_001396435.1, XP_001938589.1,
 XP_001388497.2, XP_663661.1, XP_003295335.1, XP_002152088.1, XP_001212071.1,
 Q12573.1, XP_002379858.1, XP_001821592.1, XP_002844341.1, XP_001394678.1,
 ACD75400.1, BAK03594.1, XP_003170343.1, XP_001265480.1, XP_002550661.1, EDP55514.1,
 25 XP_001528842.1, XP_749919.1, XP_001593058.1, P30612.1, EGC48494.1, EEH04429.1,
 XP_001585586.1, XP_003236182.1, XP_001400199.1, EEQ46951.1, XP_721410.1, EGP87864.1,
 XP_002380808.1, XP_001792771.1, XP_001208515.1, XP_001216161.1, XP_003071804.1,
 EFW16963.1, XP_002542118.1, XP_001936677.1, EGD95268.1, XP_003015678.1, XP_501748.1,
 XP_003169562.1, EFY96492.1, XP_682653.1, XP_002421356.1, CAK43439.1, EFY93677.1,
 30 XP_747767.1, XP_001244958.1, XP_003019635.1, XP_002847463.1, EGP83273.1, EGR52487.1,
 XP_002622526.1, XP_002563618.1, CBX99718.1, XP_001552081.1, XP_003066638.1,
 XP_003176049.1, ACD75402.1, BAA05145.1, XP_002482834.1, XP_001257501.1,
 XP_001934574.1, XP_001269972.1, XP_001587438.1, XP_001215856.1, XP_002149824.1,
 XP_001550556.1, XP_003011982.1, XP_001827121.1, XP_003233566.1, XP_003022481.1,
 35 EGR47044.1, EFQ34695.1, XP_003170005.1, BAG09241.1, XP_002796370.1, XP_003019300.1,
 XP_002563873.1, CAK40654.1, EEH19741.1, XP_003012518.1, EGD95716.1, XP_003239409.1,
 BAJ04363.1, XP_001537012.1, BAE66393.1, EGP85214.1, XP_002487227.1, AAV66104.1,
 EGE07669.1, XP_362943.2, XP_003016806.1, EFQ27388.1, XP_002384360.1, XP_002836323.1,
 XP_001274959.1, EFZ03093.1, XP_661521.1, XP_002849803.1, XP_001589398.1, AAR99474.1,
 40 XP_003189427.1, XP_001823699.1, XP_364111.1, XP_001262753.1, EFY86805.1,

XP_001390153.2, XP_002384738.1, XP_001941811.1, XP_001220831.1, XP_003296981.1,
 XP_002480829.1, BAD83681.1, XP_001827526.2, XP_369556.1, CAK38224.1, EFQ26532.1,
 XP_002562328.1, XP_001904540.1, EGO52476.1, XP_002382002.1, XP_001225874.1,
 XP_958030.2, XP_002540883.1, XP_001908957.1, XP_001559255.1, XP_364102.1,
 5 EDP48064.1, XP_365075.1, XP_381460.1, CBX95930.1, XP_003054099.1, XP_361347.2,
 XP_002846867.1, XP_001214985.1, EFQ35175.1, XP_002479062.1, XP_001908613.1,
 XP_003345380.1, EGR50567.1, XP_002479350.1, XP_001394417.2, XP_001394159.2,
 XP_002146776.1, EGP86783.1, EFX02953.1, CAK45889.1, XP_003006887.1, XP_002541427.1,
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 10 XP_003052680.1, XP_365851.1, XP_001799910.1, XP_003347175.1, XP_002565258.1,
 EGR48918.1, EGR52524.1, XP_964653.2, XP_002147083.1, XP_002843935.1, EEH19393.1,
 CAC10088.1, EEH47609.1, EEQ92528.1, XP_001246560.1, XP_002626168.1, XP_003024880.1,
 XP_003169255.1, XP_003013780.1, XP_003235691.1, XP_746816.1, EGD98483.1,
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 15 XP_001548471.1, XP_001806478.1, EFQ34989.1, XP_001552987.1, CAC24473.1,
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 XP_002628451.1, XP_001910644.1, EGR44510.1, EFQ36733.1, XP_003052472.1,
 XP_001393445.2, XP_001522438.1, EGO04179.1, XP_001397944.2, CAK49049.1, EFQ30109.1,
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 20 EGO04180.1, CAK46976.1, XP_370476.1, XP_002145942.1, XP_003004457.1, ADK36663.1,
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 XP_002378813.1, EGR46513.1, XP_003033448.1, XP_002145326.1, XP_662462.1,
 XP_747469.1, XP_001935085.1, EGR45892.1, EGO01601.1, EGP89995.1, XP_001222615.1,
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 25 ADK36661.1, EFY97845.1, XP_001834501.1, EGO03790.1, XP_001884320.1, XP_003028899.1,
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 BAK09418.1, BAK09442.1, EGO28830.1, EGE03365.1, EFZ01428.1, EGO03065.1,
 XP_001558890.1, XP_002487181.1, EGO29652.1, AAX49400.1, EFY92529.1, XP_002380252.1,
 30 XP_001884460.1, BAK09387.1, XP_001839366.2, XP_003031835.1, EFY99978.1, AAL67906.1,
 BAG09240.1, XP_002381768.1, XP_001800031.1, XP_001825073.2, BAE63940.1,
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 35 XP_001560806.1, CBF69707.1, ADN43682.1, XP_001593179.1, XP_001886909.1,
 XP_001934479.1, XP_001587730.1, XP_001886909.1, XP_001831709.2, XP_001392650.1,
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 XP_001598033.1, XP_001835239.2, EGN97256.1, XP_001554305.1, NP_182075.1,
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 40 XP_002143660.1, XP_003327130.1, BAJ78287.1, XP_002880182.1, ACB59278.1, EFQ36688.1,

BAJ78285.1, BAJ78286.1, XP_001798699.1, EEH44101.1, BAJ78288.1, BAJ78284.1, EGG02425.1, EGG03011.1, AAA34334.1, NP_001189747.1, EGG02601.1, XP_002978645.1, EGG11203.1, XP_762610.1, XP_762620.1, XP_001545581.1, CAB44684.1, CAN80536.1, AAN05337.1, NP_001049423.1, XP_001791898.1, NP_001031814.1, XP_002279531.1, 5 ABK94777.1, AAZ39646.1, XP_002880183.1, ABC68403.1, XP_002839066.1, EGG03014.1, XP_002320074.1, NP_001182854.1, CBI38795.3, XP_002310605.1, NP_196442.2, XP_002270594.1, ABZ80830.1, XP_002275905.1, CBI38796.3, XP_002476978.1, CAB93726.1, EGG03624.1, EGG06527.1, NP_197710.1, XP_001768338.1, XP_002270673.1, BAJ86572.1, XP_002275806.1, CBI38797.3, XP_002320072.1, CAN60189.1, XP_002986290.1, 10 XP_002465888.1, CAN80040.1, XP_002336104.1, XP_002988354.1, XP_002264277.1, EGD72898.1, XP_002866853.1, EAY95236.1, XP_002979701.1, XP_002988762.1, XP_002304502.1, XP_002873349.1, XP_003192947.1, CAN63571.1, NP_001053615.1, NP_176558.1, EGC49561.1, EGG09027.1, XP_002314581.1, XP_002446966.1, XP_002320802.1, ABC59095.1, XP_003323121.1, XP_002974639.1, XP_002395587.1, 15 XP_002866852.1, XP_002319770.1, NP_001146262.1, NP_001169224.1, AAM65207.1, XP_002529058.1, XP_002886391.1, XP_002320071.1, XP_002446967.1, XP_757870.1, EAY95147.1, XP_002899664.1, EEH05830.1, XP_002874114.1, ADO24345.1, BAJ88802.1, BAA05146.1, XP_002963351.1, EAY88475.1, NP_195658.3, XP_002976944.1, ABC59093.1, XP_002275114.1, XP_003328407.1, CAN75428.1, BAJ86471.1, XP_002981144.1, 20 XP_002277006.1, EAZ26110.1, ACN41008.1, XP_002899542.1, XP_001781614.1, EAY76187.1, BAK06758.1, XP_002511745.1, XP_002982626.1, XP_002963763.1, NP_001065111.1, ABF93892.1, XP_002314117.1, BAK06287.1, XP_001745327.1, NP_001047674.1, XP_002878665.1, XP_002974847.1, NP_179899.1, CAN80156.1, NP_001053543.1, ABC59094.1, XP_002328165.1, XP_002270628.1, XP_002275115.1, XP_002980688.1, XP_002465039.1, 25 AAL91155.1, NP_195910.1, XP_002509820.1, NP_200694.1, CAA62082.1, AAL75903.1, XP_002468241.1, XP_002883546.1, XP_002862636.1, XP_002312905.1, EAY79269.1, AAM12494.1, XP_002875027.1, XP_758010.1, XP_002509524.1, AAP54707.2, XP_002869292.1, NP_001143079.1, ACF82946.1, XP_002270497.1, XP_002979685.1, XP_002465041.1, XP_002533544.1, AAG17470.1, XP_002985393.1, NP_191946.1, XP_002525608.1, AAZ39642.1, 30 XP_002270428.1, XP_002529227.1, CBI24485.3, XP_001763206.1, EGG02922.1, XP_002974848.1, NP_001141467.1, CBI27149.3, NP_001130907.1, XP_002982474.1, NP_001048917.1, XP_002465889.1, ABZ80831.1, XP_002464461.1, EAY88476.1, BAJ90714.1, XP_002893825.1, ACN28568.1, XP_002452782.1, XP_002280004.1, XP_001764611.1, NP_001183394.1, BAJ89570.1, CBI24484.3, BAJ88840.1, ACG38359.1, CAN77648.1, 35 BAJ91452.1, NP_001141345.1, XP_002282185.1, XP_002980994.1, XP_002299820.1, BAJ87982.1, BAJ91842.1, XP_003325270.1, XP_001760399.1, CBI34058.3, ADG34845.1, XP_002523775.1, EEH21852.1, Q50EK3.1, BAK06748.1, XP_002963764.1, ACN34158.1, XP_001764503.1, XP_002311750.1, XP_001782495.1, XP_002988642.1, XP_002465625.1, XP_002892051.1, XP_002279649.1, NP_171666.1, ABK28430.1, BAC42067.1, AED99869.1, 40 NP_174713.1, XP_001781706.1, ABG66204.1, XP_002964775.1, NP_001064901.2,

XP_002961706.1, XP_002519477.1, XP_001559854.1, CBH32594.1, BAB92258.1,
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 BAJ89814.1, CBI38794.3, XP_776769.1, NP_001141372.1, EEC74485.1, EAY76557.1,
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 5 XP_002979336.1, BAC42841.1, BAF46296.1, XP_002306380.1, XP_002865907.1, ACG34921.1,
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 EEE53477.1, CBH32607.1, EAY94753.1, NP_001130939.1, NP_182121.1, XP_002437749.1,
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 10 XP_002456654.1, ACN27732.1, XP_002445325.1, EER40289.1, XP_001838184.2, BAJ85532.1,
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 BAJ87689.1, NP_001044838.1, XP_002521004.1, XP_002882043.1, XP_002527038.1,
 XP_002318721.1, XP_002979339.1, NP_176086.1, XP_001560028.1, ABC59092.1, ABF93891.1,
 ACR38435.1, EAY78983.1, NP_179782.1, CCA21696.1, XP_002334340.1, EFX88387.1,
 15 NP_001044554.1, XP_002321857.1, NP_173862.1, NP_195660.1, XP_001554079.1,
 EAZ13864.1, EEC67630.1, EAY76183.1, AAP54710.2, NP_001065112.2, ACD10924.1,
 XP_001559275.1, EEC67338.1, XP_002273811.1, ADJ68242.1, NP_001065698.1, CAN66874.1,
 CAB41474.1, XP_002868908.1, XP_002904660.1, CAR47816.1, NP_189243.1, EAY98229.1,
 XP_002448320.1, O81117.2, XP_002458797.1, XP_002277129.1, BAJ88829.1, CAN67559.1,
 20 BAK08034.1, XP_002894062.1, XP_002894891.1, XP_002279981.1, ABR16451.1, NP_201150.1,
 AAM60854.1, XP_002521002.1, XP_002521474.1, XP_002875311.1, NP_195661.1, AAP79889.1,
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 ABA95812.1, XP_002998647.1, NP_001066166.2, XP_002894690.1, EFY92064.1,
 XP_002278009.1, XP_002336002.1, CCA16508.1, XP_002868909.1, EAZ31703.1, C96517,
 25 EAY86526.1, XP_002307954.1, XP_002904638.1, XP_002266883.1, XP_002439880.1,
 XP_002892730.1, ADI52567.1, EGI61791.1, XP_002511196.1, EGG04372.1, XP_002511875.1,
 ACE75189.1, NP_001055681.1, XP_001589816.1, NP_001170655.1, XP_002300789.1,
 XP_001934479.1, XP_001587730.1, XP_001554079.1, XP_001559275.1, XP_002868908.1,
 XP_002998647.1, EFY92064.1, XP_002605799.1, BAC43393.1, ABK28457.1, AAL54887.1,
 30 BAC43161.1, XP_002333384.1, ZP_03631129.1, AAL84318.1, BAJ99856.1, XP_002593704.1,
 YP_001965159.1, XP_002454121.1, EFX88390.1, ABR16969.1, NP_177109.3, XP_002441724.1,
 NP_001166017.1, BAB92256.1, ACE75340.1, AAZ39645.1, XP_002312417.1, XP_002887239.1,
 NP_001172609.1, NP_001065766.1, XP_002515053.1, AAL54885.1, ABR16897.1,
 XP_002878579.1, NP_001140775.1, XP_003275955.1, ZP_08045694.1, BAJ94069.1,
 35 XP_001654558.1, XP_002436562.1, EAY88702.1, BAK03685.1, XP_003327629.1,
 XP_002322606.1, EEH42702.1, XP_002037976.1, NP_172774.1, XP_002282477.1, EFX88388.1,
 XP_002522465.1, EFZ21470.1, AAO41955.1, AAL54886.1, XP_002450277.1, XP_002862559.1,
 XP_002335046.1, XP_003328408.1, ACE75187.1, XP_001849294.1, XP_002444132.1,
 XP_002894061.1, EFN77015.1, EGI69992.1, CBI17962.3, AAL54884.1, XP_002998650.1,
 40 XP_002105150.1, XP_002877615.1, EFZ22412.1, XP_002439815.1, XP_002300790.1,

CBI40391.3, AEI59774.1, XP_002801151.1, XP_003325267.1, XP_001554577.1, EAY79865.1,
 XP_002465796.1, XP_002931035.1, ABA91371.1, ACE75338.1, XP_001592850.1,
 XP_001362981.1, XP_002271246.1, EGB11905.1, NP_176713.1, CBJ27248.1, NP_566155.1,
 EFX87732.1, EEC71661.1, ACG29046.1, NP_001130576.1, XP_001843663.1, ABK25134.1,
 5 EGI65081.1, XP_002722841.1, AAL67908.2, AAO15579.1, YP_122047.1, EFA04617.1,
 YP_001522424.1, ACB87383.1, NP_001027517.1, EEE52725.1, XP_002078257.1,
 XP_002722842.1, ZP_05128707.1, XP_003208874.1, AAK31592.1, ABA95747.2,
 NP_001181472.1, NP_001075572.1, XP_001108915.1, XP_001520882.1, XP_002063219.1,
 EFZ22408.1, AAL57721.1, EFW47740.1, AAQ20834.1, CAN74644.1, XP_002722849.1,
 10 BAC30028.1, CAN75729.1, XP_002115603.1, AAN72309.1, EEC68823.1, CAM18519.1,
 EAZ13863.1, XP_002906159.1, NP_001003947.1, ZP_01858832.1, XP_002882162.1,
 XP_002089195.1, XP_002892729.1, CAN68037.1, NP_001130648.1, NP_001166016.1,
 NP_172773.4, ADJ68241.1, EGI62551.1, EFN63658.1, XP_002300103.1, XP_001658673.1,
 XP_001367719.1, NP_775146.1, XP_001375048.1, AAH21377.1, NP_727589.1,
 15 XP_002271847.1, XP_001809620.1, XP_002897528.1, NP_190421.1, XP_002282468.1,
 XP_536868.2, EEE58297.1, XP_001992105.1, EAY82190.1, ADD20161.1, XP_001363065.1,
 EAU77129.3, EAY72807.1, EGG03077.1, NP_001181489.1, NP_001177869.1, XP_001966135.1,
 BAA99522.1, BAK07250.1, XP_002133118.1, NP_001042228.1, AAL57720.1, XP_002897529.1,
 AAA35712.1, YP_002275016.1, NP_000770.2, XP_002721578.1, XP_321208.4, AAM09532.1,
 20 EFN61085.1, BAK06179.1, EFX88389.1, YP_001602608.1, XP_513140.3, NP_001182438.1,
 AAD31068.1, NP_001093242.1, XP_001367758.2, EFZ18984.1, YP_691921.1, CAH59968.1,
 AAS80270.1, CAH59967.1, ACQ99381.2, YP_003810988.1, YP_957888.1, CBW44755.1,
 ZP_05042596.1, ZP_01913735.1, ZP_05043097.1, ADQ00145.1, YP_004494060.1,
 ZP_08206912.1, BAE78452.1, NP_114222.1, ACZ56357.1, YP_640381.1, ZP_04384919.1,
 25 ZP_08025219.1, ZP_07715822.1, ZP_06847816.1, YP_001702784.1, AEK27137.1,
 ZP_07716433.1, ZP_08199554.1, YP_004495520.1, YP_345718.1, ZP_08022914.1,
 YP_001851443.1, BAG50428.1, YP_001135848.1, BAF95905.1, YP_345695.1, ACP39691.1,
 ACP39664.1, ACP39635.1, ACP39633.1, ACP39710.1, ACP39698.1, ACP39711.1, BAE47475.1,
 BAE47474.1, ABW76858.1, ACO50699.1, ACP39643.1, ACP39639.1, ACP39708.1, ACM68663.1,
 30 ACP39642.1, ACP39684.1, ACP39636.1, ZP_05095005.1, ACP39652.1, BAE47473.1,
 ACM68664.1, ACP39646.1, ACP39680.1, ACP39692.1, ACP39675.1, ACP39632.1,
 ZP_05129284.1, ACP39706.1, ACP39695.1, ACM68665.1, ACP39654.1, ACP39665.1,
 ACP39649.1, BAE47472.1, ACM68668.1, ACP39676.1, ACP39648.1, ACP39647.1,
 ZP_01102434.1, ACM68666.1, ACP39641.1, ACM68669.1, ZP_01625037.1, ACP39690.1,
 35 ACP39696.1, ACP39697.1, ACP39707.1, ACP39682.1, ACP39650.1, ACP39638.1,
 ZP_05126641.1, CAH04396.1, ACP39658.1, ZP_01102687.1, ACJ06772.1, YP_001413041.1,
 YP_552058.1, ADE05601.1, ADI19685.1, BAE47479.1, ZP_01626700.1, ZP_01618279.1,
 CAH61448.1, YP_001411305.1, YP_003591161.1, ZP_01615522.1, ACM68667.1, ACP39651.1,
 ZP_05095535.1, ZP_01618489.1, NP_418882.1, ADI19983.1, ACP39677.1, BAE47476.1,
 40 ACP39655.1, ACP39656.1, ADI19696.1, BAE47477.1, YP_001413399.1, YP_459878.1,

- BAE47480.1, BAE47481.1, ACP39653.1, BAE47478.1, YP_001681656.1, ZP_01618281.1, ZP_01627262.1, YP_001413057.1, YP_760740.1, YP_001242466.1, YP_001203574.1, CAH61454.1, YP_002129656.1, YP_001672075.1, ACP39709.1, YP_001990805.1, NP_946959.1, YP_001203575.1, YP_783213.1, YP_003059227.1, YP_004110202.1, ACP39645.1,
- 5 YP_487538.1, CAH61451.1, YP_570816.1, YP_534107.1, YP_001413223.1, YP_001242465.1, YP_557448.1, ZP_08631162.1, NP_773883.1, ZP_00997728.1, ACP39683.1, NP_768493.1, NP_773882.1, ZP_08271781.1, CAH61449.1, YP_003883668.1, YP_003332953.1, YP_004535688.1, YP_495502.1, YP_459378.1, ZP_08700267.1, ZP_01863452.1, ZP_06860085.1, BAE47487.1, YP_617903.1, ZP_08207422.1, BAE47486.1, ZP_01041003.1,
- 10 BAE47484.1, ACR78197.1, CAH61456.1, ZP_01858113.1, ACP39681.1, BAE47485.1, ACP39673.1, BAE47483.1, ACP39669.1, BAE47482.1, ACP39674.1, ACP39704.1, ACP39703.1, YP_497095.1, ACP39672.1, ACP39702.1, ACP39670.1, ACP39666.1, YP_458852.1, ACP39687.1, ACP39688.1, ACP39634.1, ACP39686.1, ACP39660.1, ACP39700.1, YP_001411309.1, ZP_01465241.1, ACP39701.1, ACP39679.1, ACP39657.1, ACP39694.1,
- 15 ACP39659.1, ACP39671.1, ACP39693.1 and YP_003342921.1.

Enzyme E_b

In another example, the enzyme E₁ may at least be one AlkB alkane hydroxylase (E_{1b}) selected from the group consisting of:

- 20 YP_001185946.1, Q9WWW6.1, YP_957898.1, YP_957728.1, YP_694427.1, BAC98365.1, ZP_00957064.1, CAC86944.1, YP_001672212.1, CAB59525.1, ACH99213.1, ACH99215.1, ACH99216.1, AAK56792.1, ACH99229.1, ACS91348.1, AAP41820.1, ZP_05128075.1, CAM58121.1, CAM58085.1, ACQ44675.1, ACZ62808.1, ZP_01738706.1, ZP_01916228.1, ZP_01225325.1, YP_001023605.1, ACJ22747.1, ACT91140.1, AAT91722.2, CBA27418.1,
- 25 YP_001889129.1, EGC97932.1, ACT91201.1, ZP_05083049.1, YP_554098.1, ZP_01900149.1, ADG26619.1, ADG26657.1, ADG26640.1, ZP_06838771.1, ADG26649.1, ADG26651.1, ZP_02374120.1, YP_368326.1, ZP_02380481.1, ADG26643.1, ADG26628.1, YP_442346.1, ADG26620.1, ADG26647.1, ZP_07673680.1, ADG26638.1, YP_002232139.1, YP_001118743.1, ZP_01764629.1, YP_108945.1, YP_334185.1,
- 30 ZP_04897834.1, ZP_02889567.1, YP_620386.1, YP_002897546.1, ZP_02166109.1, ZP_02904755.1, ADG26639.1, YP_001892637.1, ADG26642.1, ZP_04939380.1, ZP_02464124.1, YP_102417.1, CAC36356.1, ACJ22727.1, YP_001764240.1, YP_002765609.1, YP_001945311.1, ZP_03586616.1, ACJ22665.1, ZP_03574223.1, CAC37038.1, ZP_02456517.1, YP_001807560.1, YP_002779449.1, AAK97454.1,
- 35 YP_002912304.1, ACR55689.1, YP_003397515.1, YP_004361423.1, YP_772734.1, ACJ65014.1, ACT31523.1, ACJ22750.1, ZP_07375042.1, YP_002776786.1, ACB11552.1, ZP_02363472.1, ADG26653.1, ZP_04383196.1, ZP_02356342.1, ACJ22751.1, YP_952571.1, ACU43494.1, YP_001135977.1, YP_002764193.1, YP_003855036.1, YP_004078475.1, AAK97448.1, ZP_04388098.1, ACX30747.1, ADG26632.1, ACJ22719.1,
- 40 ADO21492.1, ZP_05061580.1, ADR72654.1, ACZ65961.1, ACX30755.1, YP_001849604.1,

AAV64895.1, YP_004495037.1, YP_702497.1, YP_001069662.1, ZP_06850622.1,
BAF34299.1, CAB51024.2, YP_004008018.1, YP_003768535.1, ACJ65013.1,
ZP_07282765.1, YP_886209.1, ACJ22725.1, ZP_08155372.1, YP_004493362.1,
ZP_05228000.1, ZP_07717360.1, BAD67020.1, YP_004524245.1, ZP_07715778.1,
5 NP_217769.1, ACS91349.1, YP_960105.1, ZP_07014137.1, YP_004746682.1,
ZP_08022271.1, ACN62569.1, ADQ37951.1, YP_003647687.1, YP_003837040.1,
ADG26600.1, YP_002768905.1, ZP_08553310.1, ADG26597.1, ACJ22749.1, ADG26598.1,
YP_001704327.1, ZP_04385381.1, ZP_04751264.1, ADG26609.1, ADG26610.1,
ZP_06417258.1, ADG26607.1, ADP98338.1, YP_003275257.1, YP_004084103.1,
10 ADG26630.1, ADG26625.1, ADG26605.1, ADG26599.1, ZP_05218167.1, ADQ37950.1,
YP_921354.1, ADG26645.1, ADG26612.1, YP_004493370.1, YP_638501.1,
YP_003809668.1, NP_962298.1, ZP_04750514.1, ADG26608.1, ADT82701.1, ACJ06773.1,
YP_120833.1, ADG26618.1, ADG26602.1, ADG26623.1, ZP_04383566.1, ZP_08122407.1,
YP_004077166.1, ZP_05041651.1, ZP_04608296.1, ABU93351.2, YP_003658078.1,
15 ADQ37949.1, ADG26652.1, YP_002765850.1, AAK97447.1, CAD24434.1, CAC40954.1,
ACT91203.1, YP_120829.1, ZP_07282558.1, YP_003298195.1, YP_001851790.1,
ZP_05827357.1, ADG26633.1, CAB51020.1, YP_953908.1, ZP_07990416.1, YP_119532.1,
ZP_08442348.1, ZP_08276444.1, ZP_04661203.1, ABO12068.2, YP_001846325.1,
ADQ37952.1, ZP_08198697.1, ZP_00996652.1, YP_001707231.1, ZP_08433663.1,
20 ZP_08205256.1, YP_003732372.1, YP_906529.1, ACT91204.1, YP_001506534.1,
YP_001713880.1, YP_883357.1, YP_004525252.1, ADG26604.1, YP_001134633.1,
ZP_08195602.1, ZP_06690500.1, ZP_05826167.1, ADY81595.1, ZP_06056754.1,
AAK31348.1, YP_251715.1, ZP_08461977.1, ZP_05847237.1, YP_712218.1,
YP_001084670.1, ZP_04387164.1, YP_260041.1, YP_002873097.1, ADG26614.1,
25 AAK97446.1, YP_001280943.1, ZP_04386125.1, AAC36353.2, CCA29159.1, CAD10804.1,
CCA29151.1, CAC40953.1, CCA29161.1, ABA55770.1, AAS93604.4, CCA29173.1,
CCA29155.1, CCA29156.1, ABA55772.1, CCA29154.1, ABA55793.1, CCA29162.1,
CCA29170.1, ZP_03824539.1, CCA29166.1, CCA29136.1, ZP_06065934.1, ABB54493.1,
CCA29169.1, YP_003112137.1, CCA29127.1, CCA29148.1, CCA29160.1, ZP_06057458.1,
30 ABA55773.1, YP_004016090.1, CCA29139.1, YP_480358.1, ABA55787.1, CCA29150.1,
CCA29130.1, ZP_07775830.1, ABA55779.1, CCA29132.1, YP_003732938.1, BAB33284.1,
CCA29149.1, CCA29145.1, ABA55783.1, CCA29137.1, CCA29129.1, CCA29158.1,
CCA29176.1, CCA29142.1, CCA29144.1, BAB33287.1, CCA29133.1, CCA29140.1,
CCA29135.1, ZP_06066074.1, ZP_03823182.1, CCA29171.1, CCA29152.1, CCA29131.1,
35 ABA55780.1, CCA29163.1, CCA29143.1, CCA29153.1, YP_001580600.1, CCA29134.1,
CCA29138.1, YP_046098.1, ZP_06072466.1, ZP_05361594.1, ACU43504.1, CCA29147.1,
CCA29146.1, ZP_06061712.1, ACT91185.1, ACT91147.1, ACT91178.1, ACT91167.1,
ACT91181.1, ACT91188.1, ZP_06069784.1, ACT91205.1, ZP_06725872.1, ACT91171.1,
CCA29128.1, ABY56787.1, ADE05602.1, ACU43474.1, ACJ22718.1, ABB90688.1,
40 ACU43519.1, ABB96093.1, ACU43485.1, ACU43493.1, ABW76857.1, ACT91163.1,

ACJ22673.1, ZP_06188150.1, ACT91242.1, ACT91225.1, ACT91211.1, ACU43479.1,
 ACU43491.1, ACU43522.1, ACU43486.1, ACT91221.1, ACJ22662.1, ACU43506.1,
 ACU43487.1, ACT91259.1, AAA97866.1, ACU43502.1, YP_001252544.1, ABB96084.1,
 ACU43520.1, ACJ22668.1, ACU43503.1, ACT91230.1, ABA55777.1, ACT91231.1,
 5 ZP_01748311.1, ACJ22724.1, ACU43475.1, ACU43511.1, ACU43490.1, ZP_08330953.1,
 ACU43484.1, CBX01596.1, ACT91168.1, YP_096989.1, ACT91215.1, YP_125370.1,
 ACT91233.1, ACU43478.1, ADE05603.1, ACJ22715.1, ACU43512.1, ACT91196.1,
 ACJ22692.1, ACU43510.1, ACU43521.1, ACT91174.1, ACT91213.1, ACT91142.1,
 ACT91206.1, ACT91216.1, ACT91182.1, ACT91255.1, ACT91246.1, ACT91217.1,
 10 ACT91155.1, ACT91240.1, ACT91207.1, ACU43495.1, YP_128249.1, ACT91160.1,
 YP_004052990.1, ACT91226.1, ACU43507.1, ABO61855.1, ACT91214.1, ACT91220.1,
 YP_001188237.1, ACJ22689.1, ZP_01689499.1, YP_004379711.1, ACJ22748.1,
 ABB90683.1, ACT91223.1, ACT91235.1, ABO61786.1, ACU43508.1, ACU43492.1,
 ACT91219.1, ACT91244.1, ABO61856.1, ACT91239.1, ACU43473.1, ABO61850.1,
 15 ACT91262.1, ACT91261.1, ACT91224.1, ACU43499.1, ACU43488.1, ADO21767.1,
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 ADO21764.1, ACU43477.1, ADO21773.1, ABO61787.1, ABB96080.1, ABO61857.1,
 ACT91228.1, ABB96070.1, ADO21744.1, ACT91245.1, CAG17608.1, ADO21747.1,
 YP_001349162.1, ABK63807.1, ZP_06879583.1, NP_250216.1, ACT91234.1,
 20 ZP_01364874.1, ABO61789.1, ADO21772.1, ACU43516.1, ACU43505.1, ACU43501.1,
 ACT91236.1, ZP_07792758.1, ACZ64723.1, ADO21743.1, ADO21759.1, ACZ64752.1,
 ADO21755.1, ACD75517.1, YP_790621.1, ACB11551.1, ADO21748.1, NP_251264.1,
 ZP_01365940.1, ADO21762.1, ADO21739.1, ACU43496.1, ABO61854.1, ZP_06878434.1,
 ACU43489.1, ACU43483.1, ADO21746.1, ACT91237.1, ZP_01895378.1, ACT91164.1,
 25 ADO21736.1, ACJ22711.1, ACZ64754.1, ZP_05042146.1, ADO21688.1, ADO21648.1,
 YP_001348003.1, ADP98656.1, ADO21737.1, ADO21760.1, ADO21754.1, ADO21740.1,
 ACZ64758.1, ACU43497.1, ZP_01912185.1, ABB96111.1, ACU43482.1, ACB11549.1,
 ADO21775.1, CCA29157.1, ADO21681.1, ADO21668.1, ADO21656.1, ACU43517.1,
 ACT91165.1, ACJ22695.1, ACJ22688.1, ABB96071.1, ADO21763.1, ACT91241.1,
 30 ADO21735.1, ACB11550.1, ADO21778.1, ACT91172.1, ADO21765.1, ABB96087.1,
 CBJ30233.1, ACJ22752.1, ABB96105.1, ACB15251.1, ACJ22694.1, ACZ64741.1,
 ACZ64706.1, ABB96108.1, ACT91191.1, ABB96101.1, ABB90691.1, ACZ64745.1,
 YP_691842.1, ABB96075.1, ABB90682.1, ABB90690.1, ADO21676.1, ADO21679.1,
 ABO61768.1, YP_435857.1, ACJ22722.1, ACT91238.1, ACZ64725.1, CAC14062.1,
 35 ADO21682.1, ACZ64771.1, ACZ64718.1, ACZ64724.1, ADO21670.1, ADO21667.1,
 CAC37048.1, ACZ64708.1, ABB96092.1, ACJ22687.1, ACZ64703.1, ADO21690.1,
 ABB92364.1, ACB11547.1, ACZ64720.1, ADO21655.1, ACZ64717.1, ADO21680.1,
 ACZ64757.1, ACZ64733.1, ACT91144.1, ACU43481.1, ACT91179.1, ZP_02181409.1,
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 40 ACU43515.1, ZP_01307000.1, ABB90685.1, YP_003862088.1, ACZ64715.1, ACZ64710.1,

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 5 ADO21685.1, ABB96072.1, ACJ22736.1, ACB11540.1, ABB96091.1, ACI04540.1,
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 15 ACB11548.1, ABB90689.1, ABB96107.1, AAT46052.1, ADO21718.1, ADO21722.1,
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 35 YP_001042010.1, YP_001234392.1, YP_351510.1, ACZ64730.1, ZP_08634611.1,
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 10 ZP_01913733.1, ABI14001.1, ABM92270.1, ABI14003.1, CAH03132.1, YP_973211.1,
 ABA55797.1, YP_003578527.1, ABJ16483.1, ABJ16482.1, CBY78068.1, ACT91260.1,
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 15 ADC29546.1, AAC38359.1, ADC29541.1, XP_001020064.1, ZP_01442436.1,
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 20 ABO26088.1, ABO61811.1, ABO61783.1, CAH55827.1, ACH99232.1, ABO61828.1,
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 CAH55824.1, ZP_08484419.1, ADA71111.1, ACJ22759.1, CAH55825.1, CAH56106.1,
 CAH56099.1, CAC40957.1, ZP_05075037.1, CAH56102.1, ZP_06846296.1, ABJ16491.1,
 ZP_05067177.1, XP_001698107.1, BAH10789.1, BAH10791.1, BAH10793.1, BAH10788.1,
 ABJ16490.1, BAH10800.1, BAH10790.1, BAH10792.1, ZP_05075214.1, BAH10799.1,
 40 BAH10795.1, BAH10787.1, BAH10798.1, BAH10794.1, BAH10801.1, BAH10796.1,

BAH10797.1, BAH10802.1, CAH56095.1, CAH56096.1, ADC29538.1, ABX76425.1, ZP_06727686.1, ZP_07774883.1 and YP_001615042.1.

Enzyme E₂

- 5 Enzyme E₂ may be capable of converting a 1-alkanol to the corresponding 1-alkanal. In particular, E₂ may be at least one P450 alkane hydroxylases (E_a) of EC 1.14.15.3, AlkB alkane hydroxylases (E_b) of EC 1.14.15.3, alcohol oxidase (E_c) of EC 1.1.3.20 or alcohol dehydrogenase (E_d) of EC 1.1.1.1 or EC 1.1.1.2. More in particular, E₂ may be selected from the group consisting of P450 alkane hydroxylase (E_a), AlkB alkane hydroxylase (E_b), alcohol oxidase (E_c) of EC 1.1.3.20, AlkJ
- 10 alcohol dehydrogenase (E_{di}), and alcohol dehydrogenase (E_{dii}) of EC 1.1.1.1 or EC 1.1.1.2.

- In particular, E₂ may be an AlkB alkane hydroxylase (E_b) also known as an alkane monooxygenase. More in particular, E₂ may comprise sequence identity of at least 50% to the alkane monooxygenase from *Pseudomonas putida* GPo1 encoded by alkBGT. Even more in particular, E₂
- 15 may comprise sequence identity of at least 50% to the polypeptide YP_001185946.1. More in particular, E₂ may comprise a polypeptide with sequence identity of at least 50, 60, 65, 70, 75, 80, 85, 90, 91, 94, 95, 98 or 100% to a polypeptide YP_001185946.1.

Enzyme E_c

- 20 The alcohol oxidase (E_c) may be selected from the group consisting of:
AAS46878.1, ACX81419.1, AAS46879.1, CAB75353.1, AAS46880.1, XP_712350.1, XP_002422236.1, XP_712386.1, EEQ43775.1, XP_001525361.1, XP_001386087.1, XP_459506.2, CAB75351.1, CAB75352.1, XP_001385255.2, EDK39369.2, XP_001484086.1, XP_002618046.1, XP_002548766.1, XP_002548765.1, XP_003041566.1, XP_003328562.1,
- 25 XP_001214264.1, XP_001904377.1, XP_658227.1, XP_001591990.1, XP_753079.1, XP_002569337.1, XP_001268562.1, XP_003348911.1, EGP90120.1, XP_001389382.1, EER37923.1, XP_001264046.1, EGO58212.1, XP_001554225.1, XP_003298648.1, XP_959005.1, XP_002841296.1, XP_001940486.1, EGR52262.1, EEQ89581.1, EGD99881.1, EFQ33355.1, XP_001821106.1, XP_002622231.1, EGG03784.1, EGC44059.1, XP_003018036.1,
- 30 XP_003011696.1, EFY90752.1, XP_001227812.1, XP_758170.1, XP_001243546.1, XP_002479333.1, XP_003344707.1, EFW14100.1, XP_003071927.1, XP_003171263.1, XP_003051757.1, XP_002147053.1, EEH19591.1, EEH50473.1, XP_001792978.1, XP_387094.1, EFY98644.1, XP_002788971.1, XP_002842592.1, EFX04185.1, XP_003231449.1, XP_001729067.1, CBX94189.1, XP_001413535.1, ACF22878.1, B5WWZ9.1, XP_002994642.1,
- 35 XP_002269629.1, XP_002519938.1, XP_002982582.1, NP_001047464.1, EEC73620.1, XP_002981110.1, XP_002960521.1, NP_566729.1, XP_001541970.1, XP_002967201.1, BAK00483.1, XP_002182547.1, BAK02336.1, XP_002454190.1, XP_002328753.1, XP_002867943.1, XP_002285334.1, CAC87643.1, CAN71289.1, XP_002454188.1, AAL31049.1, XP_002464494.1, AAL31021.1, YP_117187.1, XP_002543430.1, CAA18625.1, XP_002883430.1,
- 40 NP_193673.2, XP_002529832.1, XP_001753124.1, NP_001142399.1, ACN27562.1,

XP_002464495.1, ACR36691.1, BAJ86655.1, B5WWZ8.1, NP_001148058.1, ABR17814.1,
 EAY78905.1, NP_194586.1, AAM63097.1, AAK64154.1, NP_001064839.2, XP_002869492.1,
 XP_002314488.1, AAL31024.1, ZP_06967355.1, AAP54248.2, XP_002311685.1, ACF87929.1,
 YP_907078.1, EGE07035.1, YP_001849908.1, XP_002464496.1, EEC67160.1, AAL31027.1,
 5 XP_001761391.1, XP_002961172.1, XP_002528823.1, XP_002966834.1, NP_001176205.1,
 XP_001763007.1, XP_002272123.1, XP_002889487.1, XP_003003157.1, NP_285451.1,
 EGG23219.1, NP_171895.2, YP_003395677.1, Q9ZWB9.1, ACF88407.1, ZP_06413771.1,
 EEE51131.1, YP_003835264.1, YP_003397164.1, YP_004081922.1, XP_003294587.1,
 EEE51130.1, YP_003647529.1, YP_003647985.1, CBI29206.3, XP_629786.1, ZP_07964664.1,
 10 EEE57396.1, EEH09589.1, YP_003265796.1, YP_001840752.1, ZP_08620775.1, ACR36076.1,
 ZP_05043749.1, YP_980677.1, ZP_05043728.1, YP_692894.1, NP_710223.1, EEC67159.1,
 AAP03110.1, EFA85697.1, YP_691805.1, YP_551012.1, YP_001174466.1, YP_002796294.1,
 YP_004716331.1, YP_001019547.1, YP_585737.1, AEA86007.1, YP_960830.1,
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 15 ZP_03535393.1, ZP_06801690.1, YP_001849132.1, NP_854165.1, ZP_03427234.1, CBJ27378.1,
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 YP_003167456.1, YP_606070.1, ZP_06850167.1, ADP99095.1, YP_907986.1, ZP_04924166.1,
 ZP_08139923.1, YP_001270300.1, YP_521830.1, YP_003147410.1, YP_002007173.1,
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 20 YP_003914667.1, ZP_01306356.1, ZP_04750553.1, YP_002875279.1, YP_004704374.1,
 YP_001671392.1, NP_249055.1, ZP_06876360.1, YP_001345853.1, YP_002437969.1,
 YP_004356853.1, YP_351075.1, CBI23676.3, YP_001189668.1, YP_001528881.1,
 YP_001613612.1, YP_001747218.1, YP_003393002.1, YP_001365074.1, ZP_07778129.1,
 ZP_07392715.1, YP_001553329.1, YP_262925.1, YP_751961.1, YP_564183.1, YP_003811876.1,
 25 YP_002356821.1, YP_001051828.1, YP_001837525.1, NP_716513.1, ZP_01915079.1,
 ZP_02156621.1, YP_001184631.1, YP_001475595.1, ZP_05042393.1, YP_962228.1,
 YP_001612275.1, ADV55625.1, YP_001675797.1, YP_003555260.1, ZP_01075039.1,
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 35 ZP_01546137.1, ZP_07661079.1, YP_001860640.1, ZP_06052841.1, ZP_01881170.1,
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- ZP_05912795.1, YP_003390234.1, YP_003122799.1, CCB77579.1, EGB06416.1,
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 15 ZP_03393221.1, NP_356358.1, ZP_06055780.1, YP_001684562.1, ZP_08528157.1, BAD03162.1,
 YP_001800712.1, ACL37106.1, YP_883489.1, ZP_01075202.1, NP_969446.1, ZP_01129577.1,
 YP_001530285.1, ZP_04746501.1, YP_001341980.1, YP_905003.1, ZP_05218299.1, and
 ZP_08665577.1.
- 20 In particular, the alcohol oxidase (E_c) may be selected from the group consisting of AAS46878.1,
 ACX81419.1, AAS46879.1, CAB75353.1, AAS46880.1, XP_712350.1, XP_002422236.1,
 XP_712386.1, EEQ43775.1, CAB75351.1, CAB75352.1, XP_002548766.1, and XP_002548765.1.

Enzyme E_{di}

- 25 In particular, the AlkJ alcohol dehydrogenase (E_{di}), may be selected from the group consisting of:
 Q00593.1, Q9WWW2.1, ZP_00957061.1, YP_957894.1, CAC38030.1, YP_694430.1,
 YP_957725.1, YP_001672216.1, YP_552061.1, YP_130410.1, ZP_06155535.1, ZP_01222730.1,
 YP_691907.1, YP_002297804.1, YP_004283522.1, YP_001234383.1, YP_004435031.1,
 ZP_05110316.1, ZP_05042898.1, YP_004466324.1, ZP_08553549.1, YP_004125220.1,
 30 ADI22536.1, ADI18461.1, YP_003810975.1, YP_662346.1, YP_004427557.1, YP_692606.1,
 ZP_05043291.1, YP_440752.1, ZP_02386160.1, ZP_04763547.1, ZP_02361232.1,
 YP_003376674.1, ZP_02354055.1, ZP_05085930.1, ADQ00130.1, YP_003643016.1,
 ZP_05040520.1, YP_691922.1, AAX23098.1, BAD07371.1, NP_104379.1, YP_002551960.1,
 YP_003908558.1, YP_987903.1, ZP_05785860.1, YP_004145612.1, YP_004140926.1,
 35 CAZ88300.1, ZP_05041901.1, YP_533645.1, ZP_01754259.1, CBA31223.1, YP_587542.1,
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 YP_002874799.1, ZP_03541051.1, YP_003606536.1, ZP_02887167.1, YP_001795572.1,
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 40 ZP_07374066.1, YP_001203981.1, ZP_06840259.1, ZP_01915145.1, NP_774525.1,

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 YP_001120981.1, ZP_03574970.1, YP_004234225.1, ZP_02377531.1, ZP_02149954.1,
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 ZP_00998343.1, ZP_05181994.1, YP_004107969.1, ZP_02168070.1, ZP_01750865.1,
 40 YP_574504.1, YP_004579902.1, YP_104440.1, ZP_05452167.1, ZP_05342702.1,

- YP_001862883.1, YP_004538242.1, ZP_07471513.1, ZP_05169558.1, ZP_00956995.1,
 ZP_05096699.1, YP_004610916.1, ZP_01218118.1, AAU95210.1, ZP_02405087.1,
 ZP_04890639.1, YP_352237.1, ZP_02413594.1, ZP_07474023.1, NP_541317.1,
 YP_001993222.1, ZP_08199001.1, YP_471839.1, ZP_02492080.1, ZP_04901176.1,
 5 ZP_06915396.1, ZP_07474845.1, ZP_07477743.1, YP_004152647.1, YP_004755056.1,
 ZP_05086419.1, YP_004577547.1, ACD99850.1, YP_980426.1, ZP_05457072.1, ZP_05936041.1,
 NP_700124.1, ADT85599.1, YP_110012.1, ZP_05076113.1, YP_001068288.1, ZP_02457871.1,
 ZP_01014169.1, EGE60620.1, YP_001346810.1, YP_003408795.1, YP_003769675.1,
 YP_001257876.1, EGH93583.1, ZP_01442222.1, YP_331617.1, ZP_05636703.1,
 10 YP_001594896.1, YP_002822967.1, YP_118823.1, ZP_01878717.1, ZP_07375284.1,
 YP_001371250.1, ZP_07658682.1, YP_002898825.1, ZP_01547199.1, YP_223070.1,
 ZP_05161482.1, ZP_04679742.1, YP_002778618.1, ZP_01626756.1, ZP_05101564.1,
 YP_002947374.1, NP_385053.1, YP_001328117.1, YP_004493948.1, YP_003339515.1,
 YP_004699488.1, ZP_05101969.1, YP_485352.1, ZP_01746033.1, ZP_06712293.1,
 15 ZP_01158125.1, ZP_01058616.1, ZP_05739755.1, NP_949067.1, ZP_02364657.1, YP_570690.1,
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 ZP_06794586.1, YP_001809828.1, YP_997974.1, YP_001476791.1, ZP_08635286.1,
 YP_676287.1, ZP_07308228.1, ZP_04596242.1, YP_001622726.1, NP_699590.1,
 20 ZP_01446884.1, YP_001168504.1, ZP_01616388.1, ZP_05117189.1, ZP_05876432.1,
 ADT64694.1, ZP_01754911.1, ZP_05880498.1, ZP_02360829.1, ZP_06052433.1,
 ZP_08663540.1, YP_003768966.1, ZP_02165422.1, ZP_00960985.1, ZP_07026655.1,
 YP_001753039.1, YP_371288.1, YP_002974725.1, YP_776880.1, ZP_05784963.1,
 ZP_05124380.1, YP_459030.1, ZP_05090690.1, ZP_05064893.1, ZP_02367982.1,
 25 ZP_01890564.1, NP_541848.1, ZP_00960263.1, ZP_02961617.1, YP_001242097.1, and
 ZP_05838258.1,

In particular E_{di} may be selected from the group consisting of Q00593.1, Q9WWW2.1,
 ZP_00957061.1, YP_957894.1, CAC38030.1, YP_694430.1, YP_957725.1, and YP_001672216.1.

30

Enzyme E_{dii}

The alcohol dehydrogenase (E_{dii}) may be selected from the group consisting of *AdhE*, *AdhP*, *YjgB*,
YqhD, *GldA*, *EutG*, *YiaY*, *AdhE*, *AdhP*, *YhhX*, *YahK*, *HdhA*, *HisD*, *SerA*, *Tdh*, *Ugd*, *Udg*, *Gmd*,
YefA, *YbiC*, *YdfG*, *YeaU*, *TtuC*, *YeiQ*, *YgbJ*, *YgcU*, *YgcT*, *YgcV*, *YggP*, *YgjR*, *Ylil*, *YqiB*, *YzzH*,
 35 *LdhA*, *GapA*, *Epd*, *Dld*, *GatD*, *Gcd*, *GlpA*, *GlpB*, *GlpC*, *GlpD*, *GpsA* and *YphC* from bacteria, in
 particular *E. coli*.

Enzyme E_3

Enzyme E_3 may be capable of converting at least one 1-alkanal to the corresponding alkanoic acid.

40 In particular, E_3 may be selected from the group consisting of P450 alkane hydroxylases (E_a) of EC

1.14.15.3-, AlkB alkane hydroxylases (E_b) of EC 1.14.15.3, bifunctional alcohol oxidases (E_c) of EC 1.1.3.20, bifunctional AlkJ alcohol dehydrogenases (E_{di}) or bifunctional alcohol dehydrogenases (E_{dii}) of EC 1.1.1.1 or EC 1.1.1.2, capable of oxidizing an 1-alkanol via an 1-alkanal directly to the corresponding alkanolic acid and aldehyde dehydrogenases (E_e).

5

Enzyme E_e

Enzyme E_e , an aldehyde dehydrogenase, may be capable of catalyzing the conversion of ω -oxoalkanoic acid (ester) = ω -carboxyalkanoic acid (ester).

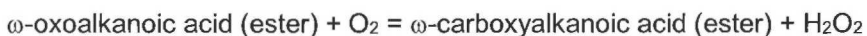
- 10 In order to catalyse the above reaction, E_e may be an aldehyde dehydrogenase of EC 1.2.1.3, EC 1.2.1.4 or EC 1.2.1.5, a fatty alcohol oxidases of EC 1.1.3.20, AlkJ alcohol dehydrogenases of EC 1.1.99.- and alcohol dehydrogenases of EC 1.1.1.1 or EC 1.1.1.2

In one example, E_e may be capable of specifically catalysing the following reaction:

- 15 ω -oxoalkanoic acid (ester) + NAD(P)⁺ = ω -carboxyalkanoic acid (ester) + NAD(P)H + H⁺

- In this case, enzyme E_e may be an aldehyde dehydrogenase of EC 1.2.1.3, EC 1.2.1.4 or EC 1.2.1.5, and may be selected from the group consisting of Prr, Usg, MhpF, AstD, GdhA, FrmA, Feab, Asd, Sad, PuuE, GabT, YgaW, BetB, PutA, PuuC, FeaB, AldA, Prr, EutA, GabD, AldB, TynA
20 and Ynel from bacteria, in particular *E. coli*.

In another example, enzyme E_e may be capable of catalysing the following reaction:



- 25 In this case, E_e may be a fatty alcohol oxidases of EC 1.1.3.20 and may be selected from the list as provided as enzyme E_c above.

In another example, E_e may be at least one AlkJ alcohol dehydrogenase of EC 1.1.99 and may be selected from the list provided above as E_{di} .

30

In a further example, E_e may be an alcohol dehydrogenases of EC 1.1.1.1 or EC 1.1.1.2 selected from the list provided as enzyme E_{dii} .

Enzyme E_4

- 35 Enzyme E_4 may be capable of converting at least one alkanolic acid to the corresponding alkanolic acid ester. In particular, E_4 may be at least one wax-ester synthase, also known as an alcohol O-acyl transferase (EC 2.3.1.20, EC 2.3.1.75) (E_f), or an alcohol O-acetyl transferase (E_g) (EC 2.3.1.20, EC 2.3.1.75 or EC 2.3.1.84).

In one example, E₄ may be at least one wax-ester synthase (E_r). More in particular, E₄ may comprise sequence identity of at least 50% to the *Acinetobacter calcoaceticus* ADP1, or O-acetyltransferase of *Hahella chejuensis*. Even more in particular, E₄ may comprise sequence identity of at least 50% to the polypeptide YP_045555.1, WP_011398768.1 or NP_808414.2. More in particular, E₄ may
 5 comprise a polypeptide with sequence identity of at least 50, 60, 65, 70, 75, 80, 85, 90, 91, 94, 95, 98 or 100% to a polypeptide selected from the group consisting of polypeptide YP_045555.1, WP_011398768.1 and NP_808414.2. In one example, E₄ may comprise a polypeptide with sequence identity of at least 50, 60, 65, 70, 75, 80, 85, 90, 91, 94, 95, 98 or 100% to SEQ ID NO:2.

10 Enzyme E_f

In particular, the Enzyme E_f may be selected from the group consisting of:

- WP_011398768.1, NP_808414.2, NP_001178653.1, XP_003272721.1, XP_002720111.1,
 NP_001002254.1, XP_529027.1, XP_002831804.1, BAC28882.1, XP_549056.2,
 XP_002918053.1, XP_001085075.1, XP_002763005.1, XP_002700092.1, XP_599558.4,
 15 EDL95940.1, XP_001496780.1, CAD89267.1, EFB28125.1, YP_004747160.1, YP_004746900.1,
 YP_004746665.1, YP_004746558.1, YP_004746531.1, YP_004746530.1, YP_004745948.1,
 YP_004745222.1, YP_004744358.1, YP_004743710.1, YP_002492297.1, AEK40846.1,
 YP_001847685.1, YP_001712672.1, YP_001706290.1, YP_004724737.1, YP_004723134.1,
 AEJ51098.1, AEJ48174.1, AEJ47480.1, YP_004392630.1, YP_004099725.1, YP_003912033.1,
 20 YP_003652731.1, YP_003301387.1, YP_003298139.1, YP_001509672.1, YP_001505948.1,
 YP_001432486.1, YP_001432432.1, YP_924893.1, YP_923981.1, YP_922869.1, YP_922597.1,
 YP_922419.1, ZP_08629145.1, ZP_08628906.1, YP_001380027.1, YP_001280731.1,
 YP_001280730.1, YP_888966.1, YP_890540.1, YP_888236.1, YP_888223.1, YP_888574.1,
 YP_884705.1, YP_889488.1, YP_886248.1, YP_882534.1, YP_881069.1, YP_881444.1,
 25 YP_883472.1, YP_879642.1, YP_884073.1, YP_880917.1, YP_882201.1, YP_879422.1,
 YP_707862.1, YP_707847.1, YP_707633.1, YP_707572.1, YP_707571.1, YP_706785.1,
 YP_706267.1, YP_705586.1, YP_705294.1, YP_702929.1, YP_701572.1, YP_700576.1,
 YP_700081.1, YP_700033.1, YP_700018.1, YP_700017.1, YP_699999.1, CCB78299.1,
 CCB78283.1, CCB72233.1, YP_004663601.1, YP_004525283.1, YP_004524901.1,
 30 YP_004524237.1, YP_004524223.1, YP_004523752.1, YP_004522677.1, YP_004521797.1,
 YP_004521441.1, YP_004020500.1, YP_004014348.1, EGO40684.1, EGO38684.1, EGO38655.1,
 EGO37244.1, EGO36970.1, EGO36701.1, YP_003951335.1, YP_003812176.1, YP_003811992.1,
 YP_003810691.1, YP_003810418.1, YP_003809501.1, ZP_08574204.1, CCA19760.1,
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 35 ZP_06271823.1, ZP_05620754.1, ZP_05360001.1, ZP_04752019.1, ZP_04751943.1,
 ZP_04750965.1, ZP_04750465.1, ZP_04750453.1, ZP_04750228.1, ZP_04750091.1,
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 ZP_04747159.1, ZP_04747093.1, ZP_04746958.1, ZP_04717323.1, ZP_04684258.1,
 40 ZP_04386203.1, ZP_04385082.1, ZP_04384030.1, ZP_04384029.1, ZP_03534755.1,

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 YP_001070856.1, YP_001069682.1, YP_001069164.1, YP_001068496.1, YP_939377.1,
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 10 YP_638030.1, YP_637968.1, YP_637380.1, YP_446603.1, NP_001185377.1, NP_200151.2,
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 ZP_05223402.1, ZP_04697793.1, ZP_02550609.1, ZP_02548969.1, EEE25493.1, ABO13188.2,
 35 ZP_07205208.1, YP_589436.1, BAJ33896.1, ZP_07718107.1, ZP_07717513.1, ZP_07717390.1,
 ZP_07716424.1, ZP_04384387.1, ZP_07376578.1, ZP_06871097.1, ZP_06852444.1,
 ZP_06852442.1, ZP_06852283.1, ZP_06852150.1, ZP_06852032.1, ZP_06850980.1,
 ZP_06850766.1, ZP_06850644.1, ZP_06849846.1, ZP_06849446.1, ZP_06849265.1,
 ZP_06848894.1, ZP_06848550.1, ZP_06847321.1, ZP_06847245.1, ZP_06728640.1,
 40 ZP_06155537.1, ZP_03822106.1, ZP_03822105.1, ZP_03264909.1, ZP_01915979.1,

ZP_01914209.1, ZP_01909198.1, ZP_01895985.1, ZP_01893763.1, ZP_01893601.1,
 ZP_01893547.1, ZP_01864269.1, ZP_01736818.1, ZP_01693481.1, ZP_01626518.1,
 ZP_01616172.1, ZP_01461648.1, ZP_01439861.1, ZP_01311414.1, ZP_01222733.1,
 ZP_01038993.1, ZP_00997001.1, ZP_06533596.1, ZP_07308012.1, ZP_07282351.1,
 5 ZP_07282257.1, ZP_07278697.1, ZP_07277986.1, ZP_07277799.1, ZP_07011797.1,
 ZP_06913634.1, ZP_06711075.1, ZP_06575037.1, ZP_06523715.1, ZP_06522644.1,
 ZP_06520408.1, ZP_06518751.1, ZP_06514733.1, ZP_06511304.1, ZP_06510466.1,
 ZP_06509700.1, ZP_06504004.1, ZP_06452618.1, ZP_06451687.1, ZP_06450049.1,
 ZP_06444722.1, ZP_06443996.1, ZP_06443677.1, ZP_06438510.1, ZP_06435077.1,
 10 ZP_06434554.1, ZP_06432969.1, ZP_06431341.1, ZP_06430915.1, ZP_05129423.1,
 ZP_05127637.1, ZP_05126217.1, ZP_05096686.1, ZP_05095013.1, ZP_05094400.1,
 ZP_05093434.1, ZP_05043539.1, ZP_05041631.1, ZP_04959394.1, ZP_04956551.1,
 ZP_01052702.1, YP_437020.1, YP_436128.1, YP_432512.1, YP_432391.1, ZP_06072118.1,
 ZP_06069021.1, ZP_06065092.1, ZP_06062254.1, YP_003032200.1, YP_003030813.1,
 15 YP_002766854.1, YP_002766842.1, YP_002766292.1, YP_002765623.1, YP_002765076.1,
 YP_002764977.1, YP_002764976.1, YP_002764693.1, YP_002764633.1, YP_002646305.1,
 YP_002646304.1, YP_001853537.1, YP_001853530.1, YP_001853214.1, YP_001852100.1,
 YP_001851711.1, YP_001851686.1, YP_001851684.1, YP_001851611.1, YP_001851610.1,
 YP_001851579.1, YP_001850950.1, YP_001850935.1, YP_001850900.1, YP_001850899.1,
 20 YP_001850378.1, YP_001849911.1, YP_001849825.1, YP_001849624.1, YP_001849470.1,
 YP_001848848.1, YP_001848784.1, YP_001822237.1, YP_001289190.1, YP_001289078.1,
 YP_001288434.1, YP_001287727.1, YP_001286168.1, YP_001085790.1, YP_856793.1,
 YP_629387.1, YP_615587.1, YP_615252.1, YP_457389.1, YP_263530.1, NP_962591.1,
 NP_962411.1, NP_962281.1, NP_961234.1, NP_960903.1, NP_960387.1, NP_960090.1,
 25 NP_959281.1, NP_959065.1, NP_857403.1, NP_857149.1, NP_857148.1, NP_857047.1,
 NP_856907.1, NP_856759.1, NP_856156.1, NP_855443.1, NP_855112.1, NP_853892.1,
 NP_828432.1, NP_603766.1, XP_003081224.1, YP_003778608.1, YP_003730939.1,
 XP_003059244.1, ADI13131.1, XP_002992800.1, XP_002963877.1, XP_001419779.1,
 XP_002988280.1, XP_002987493.1, CBH32551.1, CBH32550.1, CBH19575.1, CBH19574.1,
 30 YP_003627553.1, XP_002879777.1, XP_002877657.1, XP_002877655.1, XP_002873570.1,
 XP_002871716.1, XP_002870738.1, XP_002868506.1, XP_002865972.1, XP_002864239.1,
 XP_002862308.1, ZP_05823139.1, NP_001043877.1, ZP_06693274.1, ZP_06058985.1,
 NP_001044374.1, XP_002835451.1, XP_002787542.1, XP_002785958.1, XP_002785645.1,
 XP_002783220.1, XP_002774061.1, XP_002767852.1, XP_002766051.1, XP_002765456.1,
 35 XP_002765455.1, XP_002677788.1, XP_002671612.1, XP_002736281.1, CBA31373.1,
 XP_002184474.1, XP_002325936.1, XP_002323705.1, XP_002325937.1, XP_002323911.1,
 XP_002323706.1, XP_002328965.1, XP_002318416.1, XP_002310400.1, ACY38597.1,
 ACY38596.1, ACY38595.1, ACY38594.1, ACY38593.1, ACY38592.1, ACY38591.1, ACY38590.1,
 ACX81315.1, ACX81314.1, XP_001868729.1, XP_001847517.1, XP_001847515.1,
 40 XP_002502575.1, ACU20370.1, ACU18073.1, XP_002523348.1, XP_002516707.1,

XP_002429016.1, BAH89673.1, XP_002440221.1, XP_002459294.1, XP_002458560.1, XP_320167.4, XP_001780431.1, XP_002364905.1, XP_002263196.1, XP_002263137.1, XP_002263409.1, XP_002263252.1, XP_002268615.1, XP_002278404.1, XP_002274522.1, XP_002282418.1, XP_001633379.1, XP_001632267.1, XP_001632004.1, XP_001622638.1, 5 XP_002155609.1, XP_759225.1, XP_002152406.1, XP_001914129.1, XP_001738032.1, XP_001731626.1, XP_001209859.1, CAN79451.1, CAN78449.1, CAN72806.1, CAN71951.1, CAN71950.1, CAN76656.1, CAN62907.1, AAZ08051.1, ABO21022.1, ABO21021.1, ABO21020.1, ABJ96321.1, BAF01088.1, XP_758106.1, BAC42871.1, BAB09801.1 and BAB09102.1.

In another example, the Enzyme E_f may be selected from the group consisting of the following
10 NCBI gene identifiers:

6647910, 13882037, 13883719, 50084045, 83635736, 118163591, 118569740, 118570272, 119538589, 119959533, 126237252, 126567232, 126629771, 148572721, 148572722, 149823553, 149825234, 169147806, 196196001, 214037899, 219677786, 257447091, 262316603, 283813570, 301796553, 301796826, 311312714, 311696766, 325556018, 15 332970561, 333482117, 333482229, 333482837, 333484048, 334890574, 334890744, 353189260, 358244577, 359308666, 359732244, 359818908, 363993190, 365814880, 374845325, 377531673, 378802538, 384523048, 391857871, 391858262, 391861199, 396932954, 396935129, 399235093, 400203587, 407372801, 407812577, 432156225, 433296179, 442581482, 443888426, 444755700, 449424446, 464803513, 479864102, 20 479886236, 479966651, 480005669, 480024154, 480028610, 490485999, 498274456, 500625946, 515076064, 516264416, 516277644, 516906883, 516908681, 516909557, 516913828, 516945324, 517143888, 517432433, 517516200, 518350146, 518501601, 518568414, 518644062, 518944419, 518947555, 521014811, 521056034, 521076792, 521077398, 521090665, 521712969, 521812448, 521986522, 522129827, 522136843, 522139413 25 and 522139737.

Enzyme E_g

In particular, the Enzyme E_g may be selected from the group consisting of EGA72844.1, NP_015022.1, S69991, AAP72991.1, EDN63695.1, BAA05552.1, AAP72992.1, S69992, 30 AAP72995.1, XP_002552712.1, XP_001646876.1, XP_002551954.1, EGA82692.1, EDN61766.1, EGA86689.1, EGA74966.1, AAU09735.1, NP_011693.1, XP_445666.1, BAA13067.1, AAP72993.1, EGA62172.1, XP_455762.1, and EGA58658.1.

Enzyme E₅

35 Enzyme E₅ may be capable of converting at least one alkanolic acid ester of to the corresponding ω -hydroxy-alkanoic acid ester. In particular, E₅ may be any enzyme listed as E₁. In particular, E₅ may be at least one P450 alkane hydroxylase (E_a) of EC 1.14.15.3 or AlkB alkane hydroxylase (E_b) of EC 1.14.15.3.

40 Enzyme E₆

Enzyme E₆ may be capable of converting at least one ω -hydroxy-alkanoic acid ester to the corresponding ω -oxo alkanoic acid ester. In particular, E₆ may be any enzyme listed as E₂. In particular, E₆ may be selected from the group consisting of P450 alkane hydroxylases (E_a) of EC 1.14.15.3-, AlkB alkane hydroxylases (E_b) of EC 1.14.15.3, alcohol oxidases (E_c) of EC 1.1.3.20 and alcohol dehydrogenases (E_d) of EC 1.1.1.1 or EC 1.1.1.2.

The phrase 'when present' used in relation to Enzyme E₆, refers to cells that have been genetically modified to produce ω -oxo alkanoic acid ester. The cells according to any aspect of the present invention may comprise an increased expression of Enzyme E₆ relative to the wild type cell thus being able to produce ω -oxo alkanoic acid ester. In another example, the cells according to any aspect of the present invention may also comprise no increased expression of Enzyme E₆ relative to the wild type cell thus not being able to produce ω -oxo alkanoic acid ester. These cells may thus mainly produce ω -hydroxy-alkanoic acid ester. Therefore, when the cell according to any aspect of the present invention comprises increased expression of Enzyme E₆ (i.e. when present) then ω -oxo alkanoic acid ester may be produced.

Enzyme E₇

Enzyme E₇ may be capable of converting at least one ω -oxo alkanoic acid to the corresponding ω -amino alkanoic acid ester. In particular, E₇ may be an ω -transaminase of EC 2.6.1 (E_n).

In particular, the Enzyme E₇, may be an aminotransferase (E_n) selected from the group consisting of *Pseudomonas putida* (WP_016502144; WP_016500675.1), *Chromobacterium violaceum* (NP_901695.1), *Rhodobacter sphaeroides* 2.4.1 (YP_353455.1) and 3HMU_A, AAD41041.1, AAK15486.1, ABE03917.1, ADR60699.1, ADR61066.1, ADR62525.1, AEL07495.1, CAZ86955.1, EFW82310.1, EFW87681.1, EGC99983.1, EGD03176.1, EGE58369.1, EGH06681.1, EGH08331.1, EGH24301.1, EGH32343.1, EGH46412.1, EGH55033.1, EGH62152.1, EGH67339.1, EGH70821.1, EGH71404.1, EGH78772.1, EGH85312.1, EGH97105.1, EGP57596.1, NP_102850.1, NP_106560.1, NP_248912.1, NP_248990.1, NP_354026.2, NP_421926.1, NP_637699.1, NP_642792.1, NP_744329.1, NP_744732.1, NP_747283.1, NP_795039.1, XP_002943905.1, YP_001021095.1, YP_001059677.1, YP_001061726.1, YP_001066961.1, YP_001074671.1, YP_001120907.1, YP_001140117.1, YP_001170616.1, YP_001185848.1, YP_001188121.1, YP_001233688.1, YP_001268866.1, YP_001270391.1, YP_001345703.1, YP_001412573.1, YP_001417624.1, YP_001526058.1, YP_001579295.1, YP_001581170.1, YP_001668026.1, YP_001669478.1, YP_001671460.1, YP_001685569.1, YP_001747156.1, YP_001749732.1, YP_001765463.1, YP_001766294.1, YP_001790770.1, YP_001808775.1, YP_001809596.1, YP_001859758.1, YP_001888405.1, YP_001903233.1, YP_001977571.1, YP_002229759.1, YP_002231363.1, YP_002280472.1, YP_002297678.1, YP_002543874.1, YP_002549011.1, YP_002796201.1, YP_002801960.1, YP_002875335.1, YP_002897523.1, YP_002912290.1, YP_002974935.1, YP_003060891.1, YP_003264235.1,

YP_003552364.1, YP_003578319.1, YP_003591946.1, YP_003607814.1, YP_003641922.1,
 YP_003674025.1, YP_003692877.1, YP_003755112.1, YP_003896973.1, YP_003907026.1,
 YP_003912421.1, YP_004086766.1, YP_004142571.1, YP_004147141.1, YP_004228105.1,
 YP_004278247.1, YP_004305252.1, YP_004356916.1, YP_004361407.1, YP_004378186.1,
 5 YP_004379856.1, YP_004390782.1, YP_004472442.1, YP_004590892.1, YP_004612414.1,
 YP_004676537.1, YP_004693233.1, YP_004701580.1, YP_004701637.1, YP_004704442.1,
 YP_108931.1, YP_110490.1, YP_168667.1, YP_237931.1, YP_260624.1, YP_262985.1,
 YP_271307.1, YP_276987.1, YP_334171.1, YP_337172.1, YP_350660.1, YP_351134.1,
 YP_364386.1, YP_366340.1, YP_369710.1, YP_370582.1, YP_426342.1, YP_440141.1,
 10 YP_442361.1, YP_468848.1, YP_521636.1, YP_554363.1, YP_608454.1, YP_610700.1,
 YP_614980.1, YP_622254.1, YP_625753.1, YP_680590.1, YP_751687.1, YP_767071.1,
 YP_774090.1, YP_774932.1, YP_788372.1, YP_858562.1, YP_928515.1, YP_983084.1,
 YP_995622.1, ZP_00948889.1, ZP_00954344.1, ZP_00959736.1, ZP_00998881.1,
 ZP_01011725.1, ZP_01037109.1, ZP_01058030.1, ZP_01076707.1, ZP_01103959.1,
 15 ZP_01167926.1, ZP_01224713.1, ZP_01442907.1, ZP_01446892.1, ZP_01550953.1,
 ZP_01625518.1, ZP_01745731.1, ZP_01750280.1, ZP_01754305.1, ZP_01763880.1,
 ZP_01769626.1, ZP_01865961.1, ZP_01881393.1, ZP_01901558.1, ZP_02145337.1,
 ZP_02151268.1, ZP_02152332.1, ZP_02167267.1, ZP_02190082.1, ZP_02242934.1,
 ZP_02360937.1, ZP_02367056.1, ZP_02385477.1, ZP_02456487.1, ZP_02883670.1,
 20 ZP_03263915.1, ZP_03263990.1, ZP_03400081.1, ZP_03452573.1, ZP_03456092.1,
 ZP_03517291.1, ZP_03529055.1, ZP_03571515.1, ZP_03572809.1, ZP_03587785.1,
 ZP_03588560.1, ZP_03697266.1, ZP_03697962.1, ZP_04521092.1, ZP_04590693.1,
 ZP_04890914.1, ZP_04891982.1, ZP_04893793.1, ZP_04902131.1, ZP_04905327.1,
 ZP_04941068.1, ZP_04944536.1, ZP_04945255.1, ZP_04959332.1, ZP_04964181.1,
 25 ZP_05053721.1, ZP_05063588.1, ZP_05073059.1, ZP_05077806.1, ZP_05082750.1,
 ZP_05091128.1, ZP_05095488.1, ZP_05101701.1, ZP_05116783.1, ZP_05121836.1,
 ZP_05127756.1, ZP_05637806.1, ZP_05742087.1, ZP_05783548.1, ZP_05786246.1,
 ZP_05843149.1, ZP_05945960.1, ZP_06459045.1, ZP_06487195.1, ZP_06492453.1,
 ZP_06493162.1, ZP_06703644.1, ZP_06731146.1, ZP_06839371.1, ZP_07007312.1,
 30 ZP_07266194.1, ZP_07374050.1, ZP_07662787.1, ZP_07778196.1, ZP_07797983.1,
 ZP_08099459.1, ZP_08138203.1, ZP_08141719.1, ZP_08142973.1, ZP_08177102.1,
 ZP_08185821.1, ZP_08186468.1, ZP_08208888.1, ZP_08266590.1, ZP_08402041.1,
 ZP_08406891.1, ZP_08522175.1, ZP_08527488.1, ZP_08631252.1, ZP_08636687.1,
 In particular, the Enzyme E₇, may be an aminotransferase (E_h) selected from the group consisting
 35 of NP_901695.1, ZP_03697266.1, AAD41041.1, YP_002796201.1, ZP_03697962.1,
 YP_001859758.1, YP_002229759.1, YP_001120907.1, YP_110490.1, ZP_04964181.1,
 YP_774932.1, YP_001766294.1, YP_001581170.1, YP_622254.1, ZP_03588560.1,
 YP_001809596.1, YP_370582.1, ZP_03572809.1, NP_248990.1, YP_001888405.1,
 ZP_04905327.1, YP_001061726.1, YP_001668026.1, ZP_01750280.1, ZP_07778196.1,
 40 EGH71404.1, NP_744329.1, YP_004147141.1, ADR61066.1, ZP_05783548.1, YP_004701637.1,

YP_366340.1, YP_003264235.1, EGD03176.1, YP_001268866.1, ZP_01901558.1,
 ZP_05121836.1, YP_003692877.1, ZP_03517291.1, YP_002974935.1, YP_001668026.1,
 ADR61066.1, NP_744329.1, YP_001268866.1, YP_004701637.1, ZP_08142973.1, ADR62525.1,
 YP_610700.1, NP_747283.1, ADR62525.1, YP_001270391.1, YP_004704442.1, YP_610700.1,
 5 YP_001747156.1, ZP_08138203.1, ZP_07266194.1, EGH70821.1, YP_351134.1, EGH32343.1,
 EGH08331.1, EGH67339.1, YP_001668026.1, YP_004701637.1, YP_237931.1, ZP_03400081.1,
 ZP_05116783.1, ZP_01550953.1, ZP_07662787.1, YP_928515.1, YP_788372.1,
 YP_001021095.1, ZP_07797983.1, YP_003578319.1, YP_004305252.1, NP_248912.1,
 ZP_08636687.1, YP_003912421.1, YP_751687.1, ZP_08142973.1, YP_271307.1,
 10 ZP_05082750.1, YP_001417624.1, and YP_353455.1.

The phrase 'when present' used in relation to Enzyme E₇, refers to cells that have been genetically modified to produce ω -amino alkanolic acid ester. The cells according to any aspect of the present invention may comprise an increased expression of Enzyme E₇ relative to the wild type cell thus
 15 being able to produce ω -amino alkanolic acid ester. In another example, the cells according to any aspect of the present invention may also comprise no increased expression of Enzyme E₇ relative to the wild type cell thus not being able to produce ω -amino alkanolic acid ester. These cells may thus mainly produce ω -oxo-alkanoic acid ester. In one example, the cell comprising increased expression of Enzyme E₆ and not E₇ relative to the wild type cell may be able to produce ω -oxo-
 20 alkanolic acid ester. In another example, where the cell is not genetically modified to increase the expression of E₆ and not E₇, the cell may produce ω -hydroxy alkanolic acid ester.

Enzyme E_n

In particular, the Enzyme E_n, may be selected from the group consisting of:

25 3HMU_A, AAD41041.1, AAK15486.1, ABE03917.1, ADR60699.1, ADR61066.1, ADR62525.1, AEL07495.1, CAZ86955.1, EFW82310.1, EFW87681.1, EGC99983.1, EGD03176.1, EGE58369.1, EGH06681.1, EGH08331.1, EGH24301.1, EGH32343.1, EGH46412.1, EGH55033.1, EGH62152.1, EGH67339.1, EGH70821.1, EGH71404.1, EGH78772.1, EGH85312.1, EGH97105.1, EGP57596.1, NP_102850.1, NP_106560.1, NP_248912.1, NP_248990.1,
 30 NP_354026.2, NP_421926.1, NP_637699.1, NP_642792.1, NP_744329.1, NP_744732.1, NP_747283.1, NP_795039.1, NP_901695.1, XP_002943905.1, YP_001021095.1, YP_001059677.1, YP_001061726.1, YP_001066961.1, YP_001074671.1, YP_001120907.1, YP_001140117.1, YP_001170616.1, YP_001185848.1, YP_001188121.1, YP_001233688.1, YP_001268866.1, YP_001270391.1, YP_001345703.1, YP_001412573.1, YP_001417624.1,
 35 YP_001526058.1, YP_001579295.1, YP_001581170.1, YP_001668026.1, YP_001669478.1, YP_001671460.1, YP_001685569.1, YP_001747156.1, YP_001749732.1, YP_001765463.1, YP_001766294.1, YP_001790770.1, YP_001808775.1, YP_001809596.1, YP_001859758.1, YP_001888405.1, YP_001903233.1, YP_001977571.1, YP_002229759.1, YP_002231363.1, YP_002280472.1, YP_002297678.1, YP_002543874.1, YP_002549011.1, YP_002796201.1,
 40 YP_002801960.1, YP_002875335.1, YP_002897523.1, YP_002912290.1, YP_002974935.1,

YP_003060891.1, YP_003264235.1, YP_003552364.1, YP_003578319.1, YP_003591946.1,
 YP_003607814.1, YP_003641922.1, YP_003674025.1, YP_003692877.1, YP_003755112.1,
 YP_003896973.1, YP_003907026.1, YP_003912421.1, YP_004086766.1, YP_004142571.1,
 YP_004147141.1, YP_004228105.1, YP_004278247.1, YP_004305252.1, YP_004356916.1,
 5 YP_004361407.1, YP_004378186.1, YP_004379856.1, YP_004390782.1, YP_004472442.1,
 YP_004590892.1, YP_004612414.1, YP_004676537.1, YP_004693233.1, YP_004701580.1,
 YP_004701637.1, YP_004704442.1, YP_108931.1, YP_110490.1, YP_168667.1, YP_237931.1,
 YP_260624.1, YP_262985.1, YP_271307.1, YP_276987.1, YP_334171.1, YP_337172.1,
 YP_350660.1, YP_351134.1, YP_364386.1, YP_366340.1, YP_369710.1, YP_370582.1,
 10 YP_426342.1, YP_440141.1, YP_442361.1, YP_468848.1, YP_521636.1, YP_554363.1,
 YP_608454.1, YP_610700.1, YP_614980.1, YP_622254.1, YP_625753.1, YP_680590.1,
 YP_751687.1, YP_767071.1, YP_774090.1, YP_774932.1, YP_788372.1, YP_858562.1,
 YP_928515.1, YP_983084.1, YP_995622.1, ZP_00948889.1, ZP_00954344.1, ZP_00959736.1,
 ZP_00998881.1, ZP_01011725.1, ZP_01037109.1, ZP_01058030.1, ZP_01076707.1,
 15 ZP_01103959.1, ZP_01167926.1, ZP_01224713.1, ZP_01442907.1, ZP_01446892.1,
 ZP_01550953.1, ZP_01625518.1, ZP_01745731.1, ZP_01750280.1, ZP_01754305.1,
 ZP_01763880.1, ZP_01769626.1, ZP_01865961.1, ZP_01881393.1, ZP_01901558.1,
 ZP_02145337.1, ZP_02151268.1, ZP_02152332.1, ZP_02167267.1, ZP_02190082.1,
 ZP_02242934.1, ZP_02360937.1, ZP_02367056.1, ZP_02385477.1, ZP_02456487.1,
 20 ZP_02883670.1, ZP_03263915.1, ZP_03263990.1, ZP_03400081.1, ZP_03452573.1,
 ZP_03456092.1, ZP_03517291.1, ZP_03529055.1, ZP_03571515.1, ZP_03572809.1,
 ZP_03587785.1, ZP_03588560.1, ZP_03697266.1, ZP_03697962.1, ZP_04521092.1,
 ZP_04590693.1, ZP_04890914.1, ZP_04891982.1, ZP_04893793.1, ZP_04902131.1,
 ZP_04905327.1, ZP_04941068.1, ZP_04944536.1, ZP_04945255.1, ZP_04959332.1,
 25 ZP_04964181.1, ZP_05053721.1, ZP_05063588.1, ZP_05073059.1, ZP_05077806.1,
 ZP_05082750.1, ZP_05091128.1, ZP_05095488.1, ZP_05101701.1, ZP_05116783.1,
 ZP_05121836.1, ZP_05127756.1, ZP_05637806.1, ZP_05742087.1, ZP_05783548.1,
 ZP_05786246.1, ZP_05843149.1, ZP_05945960.1, ZP_06459045.1, ZP_06487195.1,
 ZP_06492453.1, ZP_06493162.1, ZP_06703644.1, ZP_06731146.1, ZP_06839371.1,
 30 ZP_07007312.1, ZP_07266194.1, ZP_07374050.1, ZP_07662787.1, ZP_07778196.1,
 ZP_07797983.1, ZP_08099459.1, ZP_08138203.1, ZP_08141719.1, ZP_08142973.1,
 ZP_08177102.1, ZP_08185821.1, ZP_08186468.1, ZP_08208888.1, ZP_08266590.1,
 ZP_08402041.1, ZP_08406891.1, ZP_08522175.1, ZP_08527488.1, ZP_08631252.1, and
 ZP_08636687.1.

35

The cell according to any aspect of the present invention, may be genetically modified to increase the expression relative to the wild type cells of enzymes E₁ to E₅. The cell may further be genetically modified to increase the expression of at least enzymes E₆ and/or E₇.

In one example, the enzymes E₁, E₂, E₃, E₅ and E₆, may be at least one AlkB alkane

40 hydroxylase (E_b) and Enzyme E₄ may be a wax-ester synthase (E_r). In particular,

- the AlkB alkane hydroxylase (E_b) comprises at least 60% sequence identity relative to SEQ ID NO:1; and
- the wax-ester synthase (E_f) comprises at least 60% sequence identity relative to SEQ ID NO:2.

5

In another example, when the cell according to any aspect of the present invention is genetically modified to produce at least one ω -amino alkanolic acid ester, the cell may be modified to express at least one ω -transaminase (E_h) which may comprise at least 60% sequence identity relative to SEQ ID NO:3.

10

Enzyme E_8

The cell according to any aspect of the present invention may be further genetically modified to decrease the expression of at least one enzyme E_8 that breaks down at least one of the intermediates in the process of converting alkanes to ω -functionalized carboxylic acid ester.

15

In particular, enzyme E_8 may be an enzyme capable of playing a part in the fatty acid degradation capacity of the cell. In particular, E_8 may be selected from the list consisting of acyl-CoA dehydrogenase (E_i) (FadE), enoyl CoA hydratase (E_j) (FadB), 3-hydroxyacyl-CoA dehydrogenase (E_k) (FadB) and β -ketothiolase also known as 3-ketoacyl-CoA thiolase (FadA) (E_l).

20

Fatty acids are taken up and translocated across the cell membrane *via* a transport/acyl-activation mechanism. The first intracellular step involves the conversion of acyl-CoA to enoyl-CoA through acyl-CoA dehydrogenase (E_i), the latter referred to as FadE in the case of *E. coli*. The activity of an acyl-CoA dehydrogenase may be assayed as described in the state of art, for example by monitoring the concentration of NADH spectrophotometrically at 340 nm in 100 mM MOPS, pH 7.4, 0.2 mM Enoyl-CoA, 0.4 mM NADH. The resulting enoyl-CoA is converted to 3-ketoacyl-CoA *via* 3-hydroxyacyl-CoA through hydration and oxidation, catalysed by enoyl-CoA hydratase/(*R*)-3-hydroxyacyl-CoA dehydrogenase (E_j/E_k), referred to as FadB and FadJ in *E. coli*. Enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase activity, more specifically formation of the product NADH may be assayed spectrophotometrically as described in the state of the art, for example as outlined for FadE. Finally, 3-ketoacyl-CoA thiolase (E_l), FadA and FadI in *E. coli*, catalyses the cleavage of 3-ketoacyl-CoA, to give acetyl-CoA and the input acyl-CoA shortened by two carbon atoms. The activity of ketoacyl-CoA thiolase may be assayed as described in the state of the art, for example in Antonenkov, V., et al, 1997.

35

In one example, the term "acyl-CoA dehydrogenase", as used herein, may be a polypeptide capable of catalysing the conversion of an acyl-CoA to enoyl-CoA, as part of the β -oxidation pathway. For example, the polypeptide FadE in *E. coli* (accession number: BAA77891.2) may be an acyl-CoA dehydrogenase. The term "enoyl-CoA hydratase", as used herein, also referred to as 3-hydroxyacyl-CoA dehydrogenase, refers to a polypeptide capable of catalysing the conversion of enoyl-CoA to 3-ketoacyl-CoA through hydration and oxidation, as part of the β -oxidation pathway.

40

For example, the polypeptides FadB and FadJ in *E. coli* (accession numbers: BAE77457.1 and P77399.1, respectively) are enoyl-CoA hydratases. The term "ketoacyl-CoA thiolase", as used herein, may refer to a polypeptide capable of catalysing the cleaving of 3-ketoacyl-CoA, resulting in an acyl-CoA shortened by two carbon atoms and acetyl-CoA, as the final step of the β -oxidation pathway. For example, the polypeptides FadA and FadI in *E. coli* (accession number: YP_491599.1 and P76503.1, respectively) are ketoacyl-CoA thiolases.

Enzymes E₉ and E₁₀

The cell according to any aspect of the present invention may be genetically modified further to increase the expression relative to the wild type cell of:

- the Enzyme E₉ is a fatty acyl-Coenzyme A methyl ester esterase BioH (E_m); and/or
- the Enzyme E₁₀ is a fatty acyl-Coenzyme A thioesterase (E_n) selected from the group consisting of TesA, TesB, YciA, FadM, YbfF and YbgC.

In particular, E_m may be capable of hydrolyzing fatty acid esters to free fatty acids and the respective alcohol; and/or E_n may be capable of hydrolyzing fatty acyl-Coenzyme A to free fatty acids and Coenzyme A.

Enzyme E₁₁

The cell according to any aspect of the present invention may comprise a further genetic mutation that increases the expression of at least one transporter protein relative to the wild type cell. This further mutation enables the cell to increase the uptake of at least one fatty acid. In particular, the transporter protein may be AlkL (SEQ ID NO: 4 or 5) and/or FadL (SEQ ID NO: 6). AlkL and/or FadL may function as at least one transporter protein compared to the wild type cell. In one example, the cell may be genetically modified to overexpress both the fadL and the alkL gene. The cell according to any aspect of the present invention may be genetically modified further to increase the expression relative to the wild type cell of AlkL and/or FadL.

In one example, the enzyme E₁₁ may be FadL or BAA16205.1

Enzyme E₁₂

The cell according to any aspect of the present invention may comprise a further genetic mutation that increases the expression relative to the wild type cell of an acyl-CoA synthetase (Enzyme E₁₂) of EC 6.2.1.3, EC 2.3.1.86. Enzyme E₁₂ may catalyse the conversion of a fatty acid to the CoA ester of a fatty acid, *i.e.* a molecule, wherein the functional group -OH of the carboxy group is replaced with -S-CoA. For example, the polypeptides FadD and FadK in *E. coli* (accession number: BAA15609.1 and NP_416216.4, respectively) are acyl-CoA synthetases. In another example, E₁₂ may be a long-chain-fatty-acid--CoA ligase of YP_001724804.1.

Enzyme E₁₃

Enzyme E₁₃ may be capable of converting ω -oxo alkanolic acid ester to the corresponding ω -carboxy alkanolic acid ester. In particular, Enzyme E₁₃ may be any enzyme E₃ defined above. More in particular, E₁₃ may be selected from the group consisting of P450 alkane hydroxylases (E_a) of EC 1.14.15.3-, AlkB alkane hydroxylases (E_b) of EC 1.14.15.3, bifunctional alcohol oxidases (E_c) of EC 1.1.3.20, bifunctional AlkJ alcohol dehydrogenases (E_{di}) or bifunctional alcohol dehydrogenases (E_{dii}) of EC 1.1.1.1 or EC 1.1.1.2, capable of oxidizing an ω -hydroxy alkanolic acid ester via an ω -oxo alkanolic acid ester directly to the corresponding ω -carboxy alkanolic acid ester; and aldehyde dehydrogenases (E_e).

10 **Enzyme E₁₄**

Enzyme E₁₄ may be capable of converting ω -carboxy alkanolic acid ester to the corresponding ω -carboxy alkanolic acid diester. In particular, Enzyme E₁₄ may be any enzyme E₄ defined above. More in particular, E₁₄ may be at least one wax-ester synthase (E_f) or an alcohol O-acyl transferase (E_g) (EC 2.3.1.20, EC 2.3.1.75 or EC 2.3.1.84).

15

The cell according to any aspect of the present invention does not comprise a genetic modification that increases the expression relative to the wild type cell of at least one of the following enzymes E₂₀- E₂₄ selected from the group consisting of:

- E₂₀ Acyl-ACP thioesterase, of EC 3.1.2.14 or EC 3.1.2.22,
- 20 - E₂₁ Acyl-CoA thioesterase, of EC 3.1.2.2, EC 3.1.2.18, EC 3.1.2.19, EC 3.1.2.20 or EC 3.1.2.22,
- E₂₂ Acyl-CoA:ACP transacylase,
- E₂₃ Polyketide synthase, and
- E₂₄ Hexanoic acid synthase.

25

In particular, the cell according to any aspect of the present invention has a wild type expression of enzymes E₂₀- E₂₄. Enzymes E₂₀- E₂₄ are thus neither overexpressed nor knocked out in the cells according to the method of the present invention. More in particular, the expression of any one of enzymes E₂₀- E₂₄, that is to say enzyme E₂₀, E₂₁, E₂₂, E₂₃ or E₂₄ all enzymes E₂₀, E₂₁, E₂₂, E₂₃ and
 30 E₂₄ are not genetically modified in the cell according to any aspect of the present invention. Even more in particular, the cell according to any aspect of the present invention may comprise the natural, wild type expression of any of the enzymes E₂₀-E₂₄ that may be naturally present in the cell to begin with. The cells according to any aspect of the present invention may thus be considered to comprise no recombinant expression of any one of enzymes E₂₀- E₂₄. This is especially
 35 advantageous as cells without increased expression of any one of enzymes E₂₀- E₂₄ (that is with wild type expression of any one of enzymes E₂₀- E₂₄) may then readily select to use an alkane as a carbon source for ω -functionalized carboxylic acid ester formation. In particular, any cell with increased expression of any one of enzymes E₂₀- E₂₄ may result in increased production of fatty acids which may be used as the carbon source for formation of ω -functionalised carboxylic acids
 40 and/or esters thereof by the cell with increased expression of any one of enzymes E₂₀- E₂₄. The

cells with increased expression of any one of enzymes E₂₀- E₂₄ may thus favour the use of the high concentration of fatty acids as a substrate for production of ω -functionalized carboxylic acid esters and alkanes will thus not be used for ω -functionalized carboxylic acid ester formation by the cell.

The use of other carbon sources other than alkanes for ω -functionalized carboxylic acid ester formation may increase the costs of production drastically as to produce more fatty acids, the cells would require other sources of carbon such as glucose. Accordingly, the use of cells according to any aspect of the present invention which do not comprise a genetic modification that increases the expression relative to the wild type cell of at least one of the following enzymes E₂₀- E₂₄ are used according to any aspect of the present invention for production of at least one ω -functionalized carboxylic acid ester from at least one alkane.

Enzymes E₂₀-E₂₄

Enzymes E₂₀-E₂₄ are explained in detail in WO2013024114 as enzymes E_i to E_{iv} respectively on pages 60-79 of WO2013024114.

According to another aspect of the present invention, there is provided a method of producing at least one ω -functionalized carboxylic acid ester, wherein the method comprises a step of contacting at least one cell according to any aspect of the present invention with at least one alkane. In particular, the ω -functionalized carboxylic acid ester formed may be selected from the group consisting of ω -hydroxy-alkanoic acid, ω -oxo-alkanoic acid, ω -carboxy-alkanoic acid, ω -amino-alkanoic acid esters. In particular, the ω -functionalized carboxylic acid ester may be 12-amino lauric acid methyl ester, 12-hydroxy lauric acid methyl ester, 12-carboxy lauric acid methyl (di) ester and/or lauric acid methyl ester and the alkane dodecane. In another example, the ω -functionalized carboxylic acid ester produced may be 11-amino undecanoic acid methyl ester, 11-hydroxy undecanoic acid methyl ester, 11-carboxy undecanoic acid methyl (di) ester and/or undecanoic acid methyl ester from the alkane undecane. In at least one further example, monofunctional alcohols and/or aldehydes may be formed as a by-product.

The term "contacting", as used herein, means bringing about direct contact between the alkane and/or the cell according to any aspect of the present invention in an aqueous solution. For example, the cell and the alkane may not be in different compartments separated by a barrier such as an inorganic membrane. If the alkane is soluble and may be taken up by the cell or can diffuse across biological membranes, it may simply be added to the cell according to any aspect of the present invention in an aqueous solution. In case it is insufficiently soluble, it may be solved in a suitable organic solvent prior to addition to the aqueous solution. The person skilled in the art is able to prepare aqueous solutions of alkanes having insufficient solubility by adding suitable organic and/or polar solvents. Such solvents may be provided in the form of an organic phase comprising liquid organic solvent. In one example, the organic solvent or phase may be considered liquid when liquid at 25 °C and standard atmospheric pressure. In another example, a fatty acid

may be provided in the form of a fatty acid ester such as the respective methyl or ethyl ester. In another example, the compounds and catalysts may be contacted *in vitro*, i.e. in a more or less enriched or even purified state, or may be contacted *in situ*, i.e. they are made as part of the metabolism of the cell and subsequently react inside the cell.

5

The term "an aqueous solution" is used interchangeably with the term "aqueous medium" and refers to any solution comprising water, mainly water as solvent that may be used to keep the cell according to any aspect of the present invention, at least temporarily, in a metabolically active and/or viable state and comprises, if such is necessary, any additional substrates. The person skilled in the art is familiar with the preparation of numerous aqueous solutions, usually referred to as media that may be used to keep inventive cells, for example LB medium in the case of *E. coli*. It is advantageous to use as an aqueous solution a minimal medium, i.e. a medium of reasonably simple composition that comprises only the minimal set of salts and nutrients indispensable for keeping the cell in a metabolically active and/or viable state, by contrast to complex mediums, to avoid dispensable contamination of the products with unwanted side products. For example, M9 medium may be used as a minimal medium.

20

According to another aspect of the present invention, there is provided a method of producing at least one ω -functionalized carboxylic acid ester from an alkane, wherein the method comprises:

- (a) contacting the following enzymes with the alkane:
 - (i) Enzyme E₁ capable of converting the alkane to the corresponding 1-alkanol;
 - (ii) Enzyme E₂ capable of converting the 1-alkanol of (i) to the corresponding 1-alkanal;
 - 25 (iii) Enzyme E₃ capable of converting the 1-alkanal of (ii) to the corresponding alkanoic acid;
 - (iv) Enzyme E₄ capable of converting the alkanoic acid of (iii) to the corresponding alkanoic acid ester; and
 - (v) Enzyme E₅ capable of converting the alkanoic acid ester of (iv) to the
 - 30 corresponding ω -hydroxy-alkanoic acid ester.

The method according to any aspect of the present invention may comprise a step of

(b) contacting the following enzymes with the ω -hydroxy-alkanoic acid ester:

- (vi) Enzyme E₆ capable of converting the corresponding ω -hydroxy-alkanoic acid ester of (v) to the corresponding ω -oxo alkanoic acid ester; or
- 35 (vii) Enzyme E₆ capable of converting the corresponding ω -hydroxy-alkanoic acid ester of (v) to the corresponding ω -oxo alkanoic acid ester and Enzyme E₇ capable of converting the ω -oxo alkanoic acid ester to the corresponding ω -amino alkanoic acid ester; or

- (viii) Enzyme E₆ capable of converting the corresponding ω -hydroxy-alkanoic acid ester of (v) to the corresponding ω -oxo alkanoic acid ester and Enzyme E₁₃ capable of converting the ω -oxo alkanoic acid ester to the corresponding ω -carboxy alkanoic acid ester and Enzyme E₁₄ capable of converting the ω -carboxy alkanoic acid ester to the corresponding ω -carboxy alkanoic acid diester.

The enzymes used according to any aspect of the present invention may be the same as the enzymes disclosed in the context of the cell according to the present invention.

EXAMPLES

The foregoing describes preferred embodiments, which, as will be understood by those skilled in the art, may be subject to variations or modifications in design, construction or operation without departing from the scope of the claims. These variations, for instance, are intended to be covered by the scope of the claims.

Example 1

Production of lauric acid (methyl ester) and undecanoic acid (methyl ester) from dodecane and undecane (as well as methanol in case of the methyl esters), respectively, with a whole-cell biocatalyst harbouring an alkane monooxygenase and a wax ester synthase and attenuated in an enzymes catalyzing degradation of fatty acids and an enzyme hydrolyzing fatty acid esters to free fatty acids and the respective alcohol.

Example 2

Production of 12-amino lauric acid methyl ester and 11-amino undecanoic acid methyl ester from dodecane and undecane, respectively, with a whole-cell biocatalyst harbouring an alkane monooxygenase, a wax ester synthase and an ω -transaminase and attenuated in an enzymes catalyzing degradation of fatty acids and an enzyme hydrolyzing fatty acid esters to free fatty acids and the respective alcohol.

Example 3

Construction of an expression vector for overexpression of the E. coli fadD gene and an Hahella chejuensis gene encoding a wax ester synthase

The vector pCDF-fadD_Ec-wes_Hche (SEQ ID NO:7) harbors the genes *fadD* from *E. coli* (encoding an acyl-CoA synthase) and a wax ester synthase gene from *Hahella chejuensis* (SEQ ID NO:2), codon-optimized for expression in *E. coli*. While the acyl-CoA synthase is responsible for activation of fatty acids to the corresponding CoA thioesters, the wax ester synthase is required for ester formation between a fatty acyl-CoA and an alcohol, more specifically methanol. The vector is based on plasmid pCDFDuet-1 (Merck Biosciences; Nottingham, UK) and harbors the *fadD* gene

under control of the *tac* promoter and the *Hahella chejuensis* wax ester synthase-encoding gene under control of the T5 promoter. *E. coli fadD* as well as the *tac* and T5 promoter cassettes were amplified by PCR from genomic DNA of *E. coli* W3110, respectively, the *Hahella chejuensis* wax ester synthase-encoding gene was obtained by DNA synthesis. The vector backbone and the four
 5 DNA fragments representing *E. coli fadD*, the *tac* and T5 promoter cassettes and the *Hahella chejuensis* wax ester synthase-encoding gene were fused using a commercially available kit for *in vitro* recombination (NEBuilder HiFi DNA Assembly Cloning Kit; NEB; Frankfurt/ Main, Germany) to give vector pCDF-fadD_Ec-wes_Hche (SEQ ID NO:7).

10 Example 4

Construction of E. coli strains capable of converting alkanes to the corresponding ω -functionalized fatty acid methyl esters

The expression vector pBT10_alkL (see Example 1 of WO/2011/131420 for construction details and the listed SEQ ID NO: 8) contains the genes *alkB*, *alkG*, *alkT*, *alkS* and *alkL* from
 15 the *alk* operon of *Pseudomonas oleovorans*. The corresponding gene products catalyzed oxidation of alkanes to the corresponding alkanols, alkanals and fatty acids (AlkBGT) as well as the uptake of the substrates (AlkL). In addition, the AlkBGT gene products also catalyzed the oxidation of fatty acid methyl esters, once formed by action of the enzymes acyl-CoA synthetase and wax ester synthase from fatty acids and methanol (see Example 1). The
 20 vector pJ294_alaDH_B.s._TA_C.v.(Ct) (see Example 1 of WO/2013/024114 for construction details and the listed SEQ ID NO: 17) harbors the genes *ald* from *Bacillus subtilis* (encoding an alanine dehydrogenase) and Cv_2505 from *Chromobacterium violaceum* (encoding an ω -transaminase). While the ω -transaminase is responsible for conversion of OLAME and OUAME to the corresponding amines ALAME and AUAME, alanine dehydrogenase was
 25 required for provision of the amine donor alanine from pyruvate and inorganic ammonia.

The plasmids pBT10_alkL and pCDF-fadD_Ec-wes_Hche plus when appropriate pJ294_alaDH_B.s._TA_C.v.(Ct) were transformed via electroporation into *E. coli* W3110 Δ bioH Δ fadE, plated onto LB agar plates with kanamycin (50 μ g/ml), ampicillin (100 μ g/ml) and spectinomycin (100 μ g/ml) as applicable. Transformants were screened for presence and
 30 authenticity of the plasmids by plasmid preparation and restriction digest analysis. The following strains were generated:

- *E.coli* W3110 Δ bioH Δ fadE pBT10_alkL / pCDF-fadD_Ec-wes_Hche
- *E.coli* W3110 Δ bioH Δ fadE pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pCDF-fadD_Ec-wes_Hche

35 Example 5

Biotransformation for conversion of alkanes to the corresponding ω -functionalized fatty acid methyl esters

The strains *E. coli* W3110 Δ bioH Δ fadE pBT10_alkL / pCDF-fadD_Ec-wes_Hche and *E. coli* W3110 Δ bioH Δ fadE pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pCDF-fadD_Ec-wes_Hche were subjected to fed-batch fermentation followed by biotransformation in order to investigate their ability to produce omega-hydroxylauric acid methylester (HLAME),

5 omega-oxolauric acid methylester (OLAME), omega-aminolauric acid methylester (ALAME), dodecanedioic acid monomethylester (DDAME) and dodecanedioic acid dimethylester (DDADME) from dodecane. The strains were also subjected to a fed-batch fermentation followed by biotransformation in order to investigate their ability to produce omega-

10 hydroxyundecanoic acid methylester (HUAME), omega-oxoundecanoic acid methylester (OUAME), omega-aminoundecanoic acid methylester (AUAME), undecanedioic acid monomethylester (UDAME) and undecanedioic acid dimethylester (UDADME) from undecane. This was carried out in an 8-fold parallel fermentation system from DASGIP.

For the fermentation, 1 l reactors were used which were equipped with overhead stirrers and impeller turbines. To monitor the process, pH and pO₂ were measured online. OTR/CTR

15 measurements served inter alia for estimating the metabolic activity and fitness of the cells.

The pH probes were calibrated by means of a two-point calibration with measurement solutions of pH 4.0 and pH 7.0 according to technical references provided by DASGIP. The reactors were prepared according to technical references provided by DASGIP with the required sensors and connections and the stirrer shaft was installed. The reactors were then

20 filled with 300 ml of water and autoclaved for 20 min at 121°C in order to ensure sterility. The pO₂ probes were polarized overnight (at least 6 h) following connection to the measurement amplifier. The water was then removed under the clean bench and replaced by high-cell-density medium consisting of (NH₄)₂SO₄ 1.76 g/l, K₂HPO₄ 19.08 g/l, KH₂PO₄ 12.5 g/l, yeast extracts 6.66 g/l, trisodium citrate dihydrate 11.2 g/l, 17 ml/l of a filter-sterilized 1% strength

25 ammonium iron citrate solution, and 5 ml/l of a filter-sterilized trace element stock solution (consisting of HCl (37%) 36.50 g/l, MnCl₂*4 H₂O 1.91 g/l, ZnSO₄*7 H₂O 1.87 g/l, ethylenediaminetetraacetic acid dihydrate 0.84 g/l, H₃BO₃ 0.30 g/l, Na₂MoO₄*2 H₂O 0.25 g/l, CaCl₂*2 H₂O 4.70 g/l, FeSO₄*7 H₂O 17.80 g/l, CuCl₂*2 H₂O 0.15 g/l) with 15 g/l glucose as carbon source (added by metered addition of 30 ml/l of a sterile feed solution consisting of

30 500 g/l glucose, 1% (w/v) MgSO₄*7 H₂O and 2.2% (w/v) NH₄Cl) with 50 mg/l kanamycin.

Subsequently, the pO₂ probes were calibrated using a single-point calibration (stirrer: 600 rpm / gassing: 10 sL/h air) to 100% and the feed, correction agent and induction agent stretches were cleaned by means of cleaning-in-place according to technical references provided by DASGIP. For this, the tubes were firstly flushed with 70% ethanol, then with 1 M

35 NaOH, subsequently with sterile demineralized water and finally filled with the respective media.

All of the aforementioned *E. coli* strains were cultured firstly from a cryoculture in LB medium (25 ml in a 100 ml baffled shake flask) with 50 mg/l kanamycin overnight at 37°C and 200

rpm for about 18 h. Then, 2 ml of this culture were transferred for a second preculture stage into 25 ml of high-cell-density medium consisting of $(\text{NH}_4)_2\text{SO}_4$ 1.76 g/L, K_2HPO_4 19.08 g/l, KH_2PO_4 12.5 g/l, yeast extract 6.66 g/l, trisodium citrate dihydrate 11.2 g/l, 17 ml/l of a filter-sterilized 1% strength ammonium iron citrate solution, and 5 ml/l of a filter-sterilized trace element stock solution (consisting of HCl (37%) 36.50 g/l, $\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$ 1.91 g/l, $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$ 1.87 g/l, ethylenediaminetetraacetic acid dihydrate 0.84 g/l, H_3BO_3 0.30 g/l, $\text{Na}_2\text{MoO}_4 \cdot 2 \text{H}_2\text{O}$ 0.25 g/l, $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$ 4.70 g/l, $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ 17.80 g/l, $\text{CuCl}_2 \cdot 2 \text{H}_2\text{O}$ 0.15 g/l) with 15 g/l glucose as carbon source (added by metered addition of 30 ml/l of a sterile feed solution consisting of 500 g/l glucose, 1% (w/v) $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ and 2.2% (w/v) NH_4Cl) with the already described antibiotics in a 100 ml shake flask and incubated at 37°C/200 rpm for a further 6 h.

In order to inoculate the reactors with an optical density of 0.1, the OD_{600} of the second preculture stage was measured and the amount of culture required for the inoculation was calculated. The required amount of culture was added with the help of a 5 ml syringe through a septum into the heat-treated and aerated reactor.

The following standard program was used:

DO regulator		pH regulator	
Preset	0%	Preset	0 ml/h
P	0.1	P	5
Ti	300 s	Ti	200 s
min	0%	min	0 ml/h
max	100%	max	40 ml/h

N (Rotation)	from	to	XO ₂ (gas mixture)	from	to	F (gas flow rate)	from	to
	0%	30%		0%	100%		15%	80%
growth and biotransformation	400 rpm	1500 rpm	growth and biotransformation	21%	21%	growth and biotransformation	6 sL/h	72 sL/h

Script	
Trigger sharp	31% DO (1/60h)
Induction DCPK	10 h after feed start
Feed trigger	50% DO
Feed rate	3 [ml/h]

The pH was regulated to pH 6.8 on one side with 12.5% strength ammonia solution. During cultivation and biotransformation, the dissolved oxygen (pO_2 or DO) in the culture was regulated to at least 30% by means of stirrer feed and gassing rate. Following inoculation, the DO dropped from 100% to 30%, where it was kept stable for the remainder of the fermentation.

The fermentation was carried out as fed-batch, where the feed start was triggered as delivery to the feed phase with 5 g/l*^h glucose feed, consisting of 500 g/l glucose, 1% (w/v) MgSO₄*7 H₂O and 2.2% (w/v) NH₄Cl, via the DO peak inducing the end of the batch phase. With feed start, the temperature of 37°C was lowered to 30°C. 10 h after feed start, the

5 expression of the oxidation genes was induced with 0.025% (v/v) DCPK. The start of the production (= start of the biotransformation) was carried out 14 h after feed start. For this purpose, 150 ml of dodecane or undecane were added as batch to the fermentation broth.

To quantify LSME and HLS in fermentation samples, samples were taken 1/2/4/20/22 h after the start of biotransformation. These samples were prepared for analysis as provided in

10 Example 6.

Example 6

LC-ESI/MS²-based quantification of products

The quantification of HLAME, OLAME, ALAME, DDAME and DDADME as well as of HUAME, OUAME, AUAME, UDAME and UDADME in fermentation samples was carried out

15 by means of LC-ESI/MS² by reference to an external calibration for all analytes (0.1 – 50 mg/l) and using the internal standard aminoundecanoic acid (AUA for HLSME), and d3-LSME (for LSME).

The following instruments were used here:

- 20 • HPLC system 1260 (Agilent; Böblingen) with autosampler (G1367E), binary pump (G1312B) and column oven (G1316A)
- Mass spectrometer TripelQuad 6410 (Agilent; Böblingen) with ESI source
- HPLC column: Kinetex C18, 100 x 2.1 mm, particle size: 2.6 µm, pore size 100 Å (Phenomenex; Aschaffenburg)
- 25 • Precolumn: KrudKatcher Ultra HPLC In-Line Filter; 0.5 µm filter depth and 0.004 mm internal diameter (Phenomenex; Aschaffenburg)

The samples were prepared by pipetting 1900 µl of solvent (80% (v/v) acetonitrile, 20% double-distilled H₂O (v/v), + 0.1% formic acid) and 100 µl sample in a 2-ml reaction vessel. The mixture was vortexed for about 10 seconds and then centrifuged at about 13 000 rpm for 5 min. The clear supernatant was removed using a pipette and, after appropriate dilution,

30 analyzed with diluents (80% (v/v) ACN, 20% double-distilled. H₂O (v/v), + 0.1% formic acid). 100 µL of ISTD were pipetted into each 900 µL sample (10 µL for a sample volume of 90 µL).

The HPLC separation was carried out with the aforementioned column and precolumn. The injection volume was 0.7 µL, the column temperature 50°C, the flow rate 0.6 mL/min. The

35 mobile phase consisted of eluent A (0.1% (v/v) aqueous formic acid) and eluent B (acetonitrile with 0.1% (v/v) formic acid). The following gradient profile was used:

Time [min]	Eluent A [%]	Eluent B [%]
0	77	23
0.3	77	23
0.4	40	60
2.5	40	60
2.6	2	98
5.5	2	98
5.6	77	23
9	77	23

The ESI-MS₂ analysis was carried out in positive ionization mode with the following parameters of the ESI source:

- Gas temperature 280°C
- 5 • Gas flow rate 11 L/min
- Nebulizing pressure 50 psi
- Capillary voltage 4000 V

- 10 The detection and quantification of the compounds HLAME, OLAME, ALAME, DDAME, DDADME, HUAME, OUAME, AUAME, UDAME and UDADME was carried out with the following MRM parameters, with in each case a product ion being used as qualifier and one as quantifier.

Analyte	Precursor ion [m/z]	Product ion [m/z]	Hold-up time [ms]	Collision energy [eV]
DDSME	245,2	167,1	25	6
DDSME	245,2	149,1	50	8
HLSME	231,3	181,2	15	2
HLSME	231,3	163,2	25	5
DDS	231,2	213,2	50	0
DDS	231,2	149,1	25	9
ALSME	230,3	198,1	25	10
ALSME	230,3	163,2	15	10
OLSME	229,2	197,2	50	0
OLSME	229,2	161,1	25	5
HLS	217,2	181,2	35	0

HLS	217,2	163,1	20	4
OLS	215,2	161,2	25	0
OLS	215,2	95,2	60	13

Table 1. The analytes LA und LAME were detected in SIM modus (m/z 201 and 215).

Example 7

Conversion of alkanes to the corresponding ω -functionalized fatty acid methyl esters by *E. coli* W3110 $\Delta bioH \Delta fadE$ pBT10_alkL / pCDF-fadD_Ec-wes_Hche and *E. coli* W3110 $\Delta bioH \Delta fadE$ pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pCDF-fadD_Ec-wes_Hche

Using the above described protocols *E. coli* W3110 $\Delta bioH \Delta fadE$ pBT10_alkL / pCDF-fadD_Ec-wes_Hche could be shown to produce DDAME and DDADME from dodecane as well as UDAME and UDADME from undecane (see Tables 1 and 2). Moreover, *E. coli* W3110 $\Delta bioH \Delta fadE$ pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pCDF-fadD_Ec-wes_Hche could be shown to produce HLAME and ALAME from dodecane as well as HUAME and AUAME from undecane (see Tables 2 and 3).

Strain	C HLAME [mg * L ⁻¹]	C OLAME [mg * L ⁻¹]	C ALAME [mg * L ⁻¹]	C DDAME [mg * L ⁻¹]	C DDADME [mg * L ⁻¹]
Strain 1	ND	ND	ND	50	24
Strain 2	50	ND	60	ND	12

Table 2. Concentration of ω -functionalized fatty acid methyl esters formed from dodecane with strains *E. coli* W3110 $\Delta bioH \Delta fadE$ pBT10_alkL / pCDF-fadD_Ec-wes_Hche (Strain 1) and *E. coli* W3110 $\Delta bioH \Delta fadE$ pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pCDF-fadD_Ec-wes_Hche (Strain 2).

Strain	C HUAME [g * L ⁻¹]	C OLAME [g * L ⁻¹]	C AUAME [g * L ⁻¹]	C DUAME [g * L ⁻¹]	C DUADME [g * L ⁻¹]
Strain 1	10	ND	ND	50	45
Strain 2	30	ND	10	ND	tbd

Table 3. Concentration of ω -functionalized fatty acid methyl esters formed from undecane with strains *E. coli* W3110 $\Delta bioH \Delta fadE$ pBT10_alkL / pCDF-fadD_Ec-wes_Hche (Strain 1) and *E. coli* W3110 $\Delta bioH \Delta fadE$ pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pCDF-fadD_Ec-wes_Hche (Strain 2).

201500273_Sequenzprotokoll Nachanmeldung
SEQUENCE LISTING

<110> Evonik Degussa GmbH

<120> BIOTECHNOLOGICAL PRODUCTION OF - FUCTIONALISED CARBOXYLIC ACIDS
AND ESTERS THEREOF

<130> 201500273EP

<150> EP15196180

<151> 2015-11-25

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<170> PatentIn version 3.5

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Gly Leu Val Leu Leu Val Trp Tyr Gly Ala Leu Pro Leu Leu Asp Ala
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Tyr Gly His Phe Phe Ile Glu His Asn Lys Gly His His Arg Asp Val
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Gln Lys Pro His His Ser Trp Asn Ser Asn His Ile Val Ser Asn Leu
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Val Leu Phe His Leu Gln Arg His Ser Asp His His Ala His Pro Thr
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201500273_Sequenzprotokoll Nachanmeldung

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201500273_Sequenzprotokoll Nachanmeldung

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201500273_Sequenzprotokoll Nachanmeldung

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201500273_Sequenzprotokoll Nachanmeldung

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201500273_Sequenzprotokoll Nachanmeldung

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201500273_Sequenzprotokoll Nachanmeldung

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201500273_Sequenzprotokoll Nachanmeldung

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201500273_Sequenzprotokoll Nachanmeldung

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CLAIMS

1. A microbial cell for producing at least one ω -functionalized carboxylic acid ester from at least one alkane, wherein the cell is genetically modified to increase the expression relative to the wild type cell of
 - (i) Enzyme E₁ capable of converting the alkane to the corresponding 1-alkanol;
 - (ii) Enzyme E₂ capable of converting the 1-alkanol of (i) to the corresponding 1-alkanal;
 - (iii) Enzyme E₃ capable of converting the 1-alkanal of (ii) to the corresponding alkanoic acid;
 - (iv) Enzyme E₄ capable of converting the alkanoic acid of (iii) to the corresponding alkanoic acid ester; and
 - (v) Enzyme E₅ capable of converting the alkanoic acid ester of (iv) to the corresponding ω -hydroxy-alkanoic acid ester,
 and wherein the cell does not comprise a genetic modification that increases the expression relative to the wild type cell of at least one of the following enzymes E₂₀- E₂₄ selected from the group consisting of:
 - E₂₀ Acyl-ACP thioesterase, of EC 3.1.2.14 or EC 3.1.2.22,
 - E₂₁ Acyl-CoA thioesterase, of EC 3.1.2.2, EC 3.1.2.18, EC 3.1.2.19, EC 3.1.2.20 or EC 3.1.2.22,
 - E₂₂ Acyl-CoA:ACP transacylase,
 - E₂₃ Polyketide synthase, and
 - E₂₄ Hexanoic acid synthase.

2. The cell according to claim 1, wherein the cell is further genetically modified to increase the expression relative to the wild type cell of
 - (vi) Enzyme E₆ capable of converting the corresponding ω -hydroxy-alkanoic acid ester of (v) to the corresponding ω -oxo alkanoic acid ester; or
 - (vii) Enzyme E₆ capable of converting the corresponding ω -hydroxy-alkanoic acid ester of (v) to the corresponding ω -oxo alkanoic acid ester and Enzyme E₇ capable of converting the ω -oxo alkanoic acid ester to the corresponding ω -amino alkanoic acid ester; or
 - (viii) Enzyme E₆ capable of converting the corresponding ω -hydroxy-alkanoic acid ester of (v) to the corresponding ω -oxo alkanoic acid ester and Enzyme E₁₃ capable of converting the ω -oxo alkanoic acid ester to the corresponding ω -carboxy alkanoic acid ester and Enzyme E₁₄ capable of converting the ω -carboxy alkanoic acid ester to the corresponding ω -carboxy alkanoic acid diester.

3. The cell according to either claim 1 or 2, wherein
 - the Enzyme E₁ is selected from the group consisting of P450 alkane hydroxylases (E_a) of EC 1.14.15.3- and AlkB alkane hydroxylases (E_b) of EC 1.14.15.3;
 - the Enzyme E₂ is selected from the group consisting of P450 alkane hydroxylases (E_a) of EC 1.14.15.3-, AlkB alkane hydroxylases (E_b) of EC 1.14.15.3, alcohol oxidases (E_c) of EC 1.1.3.20 and alcohol dehydrogenases (E_d) of EC 1.1.1.1 or EC 1.1.1.2;
 - the Enzyme E₃ is selected from the group consisting of P450 alkane hydroxylases (E_a) of EC 1.14.15.3-, AlkB alkane hydroxylases (E_b) of EC 1.14.15.3, aldehyde dehydrogenases (E_e), bifunctional alcohol oxidases (E_c) of EC 1.1.3.20, bifunctional AlkJ alcohol dehydrogenases (E_{di}) and bifunctional alcohol dehydrogenases (E_{dii}) of EC 1.1.1.1 or EC 1.1.1.2, wherein E_c, E_{di}, and E_{dii} are capable of oxidizing an ω -hydroxy alkanoic acid ester directly to the corresponding ω -carboxy alkanoic acid ester;
 - the Enzyme E₄ is selected from the group consisting of wax-ester synthases (E_f) of EC 2.3.1.75 or alcohol O-acyl transferases (E_g) of EC 2.3.1.84;
 - the Enzyme E₅ is selected from the group consisting of P450 alkane hydroxylases (E_a) of EC 1.14.15.3- and AlkB alkane hydroxylases (E_b) of EC 1.14.15.3;
 - the Enzyme E₆ is selected from the group consisting of P450 alkane hydroxylases (E_a) of EC 1.14.15.3-, AlkB alkane hydroxylases (E_b) of EC 1.14.15.3, alcohol oxidases (E_c) of EC 1.1.3.20, aldehyde dehydrogenases (E_e) and alcohol dehydrogenases (E_d) of EC 1.1.1.1 or EC 1.1.1.2;
 - when present the Enzyme E₇ is an ω -transaminase (E_h) EC 2.6.1;
 - when present the Enzyme E₁₃ is selected from the group consisting of P450 alkane hydroxylases (E_a) of EC 1.14.15.3-, AlkB alkane hydroxylases (E_b) of EC 1.14.15.3, aldehyde dehydrogenases (E_e), bifunctional alcohol oxidases (E_c) of EC 1.1.3.20, bifunctional AlkJ alcohol dehydrogenases (E_{di}) and bifunctional alcohol dehydrogenases (E_{dii}) of EC 1.1.1.1 or EC 1.1.1.2, wherein E_c, E_{di}, and E_{dii} are capable of oxidizing an ω -hydroxy alkanoic acid ester via an ω -oxo alkanoic acid ester directly to the corresponding ω -carboxy alkanoic acid ester; and
 - when present the Enzyme E₁₄ is selected from the group consisting of wax-ester synthases (E_f) of EC 2.3.1.75 or alcohol O-acyl transferases (E_g) of EC 2.3.1.84.
4. The cell according to any one of the preceding claims, wherein the Enzymes E₁, E₂, E₃, E₅ and E₆, is at least one AlkB alkane hydroxylase (E_b) and Enzyme E₄ is a wax-ester synthase (E_f).
5. The cell according to claim 4, wherein
 - the AlkB alkane hydroxylase (E_b) comprises at least 60% sequence identity relative to SEQ ID NO:1;

- the wax-ester synthase (E_r) comprises at least 60% sequence identity relative to SEQ ID NO:2; and
- when present the ω -transaminase (E_h) comprises at least 60% sequence identity relative to SEQ ID NO:3.

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6. The cell according to any one of the preceding claims, wherein the cell is further genetically modified to increase the expression relative to the wild type cell of at least one acyl-CoA synthetase (E_{12}) of EC 6.2.1.3 or EC 2.3.1.86.

- 10 7. A method of producing at least one ω -functionalized carboxylic acid ester, wherein the method comprises a step of contacting at least one cell according to any one of claims 1 to 6 with at least one alkane.

8. The method according to claim 7, wherein

- 15 - the ω -functionalized carboxylic acid ester is 12-amino lauric acid methyl ester, 12-hydroxy lauric acid methyl ester, 12-carboxy lauric acid methyl (di) ester and/or lauric acid methyl ester from the alkane dodecane; and/or
- the ω -functionalized carboxylic acid ester is 11-amino undecanoic acid methyl ester, 11-hydroxy undecanoic acid methyl ester, 11-carboxy undecanoic acid methyl (di) ester and/or undecanoic acid methyl ester from the alkane undecane.
- 20

9. A method of producing at least one ω -functionalized carboxylic acid ester from an alkane, wherein the method comprises:

(a) contacting the following enzymes with the alkane:

- 25 (i) Enzyme E_1 capable of converting the alkane to the corresponding 1-alkanol;
- (ii) Enzyme E_2 capable of converting the 1-alkanol of (i) to the corresponding 1-alkanal;
- (iii) Enzyme E_3 capable of converting the 1-alkanal of (ii) to the corresponding alkanoic acid;
- 30 (iv) Enzyme E_4 capable of converting the alkanoic acid of (iii) to the corresponding alkanoic acid ester; and
- (vi) Enzyme E_5 capable of converting the alkanoic acid ester of (iv) to the corresponding ω -hydroxy-alkanoic acid ester.

- 35 10. The method according to claim 9, wherein the method comprises

(b) contacting the following enzymes with the ω -hydroxy-alkanoic acid ester:

- (vi) Enzyme E_6 capable of converting the corresponding ω -hydroxy-alkanoic acid ester of (v) to the corresponding ω -oxo alkanoic acid ester; or

- (vii) Enzyme E₆ capable of converting the corresponding ω -hydroxy-alkanoic acid ester of (v) to the corresponding ω -oxo alkanoic acid ester and Enzyme E₇ capable of converting the ω -oxo alkanoic acid ester to the corresponding ω -amino alkanoic acid ester; or
- 5 (viii) Enzyme E₆ capable of converting the corresponding ω -hydroxy-alkanoic acid ester of (v) to the corresponding ω -oxo alkanoic acid ester and Enzyme E₁₃ capable of converting the ω -oxo alkanoic acid ester to the corresponding ω -carboxy alkanoic acid ester and Enzyme E₁₄ capable of converting the ω -carboxy alkanoic acid ester of (vi) to the corresponding ω -carboxy alkanoic acid diester.
- 10
11. The method according to either claim 9 or 10, wherein
- the Enzyme E₁ is selected from the group consisting of P450 alkane hydroxylases (E_a) and AlkB alkane hydroxylases (E_b) of EC 1.14.15.3;
 - 15 - the Enzyme E₂ is selected from the group consisting of P450 alkane hydroxylases (E_a), AlkB alkane hydroxylases (E_b) of EC 1.14.15.3, alcohol oxidases (E_c) and alcohol dehydrogenases (E_d);
 - the Enzyme E₃ is selected from the group consisting of P450 alkane hydroxylases (E_a) of EC 1.14.15.3-, AlkB alkane hydroxylases (E_b) of EC 1.14.15.3, aldehyde dehydrogenases (E_e), bifunctional alcohol oxidases (E_c) of EC 1.1.3.20,
 - 20 bifunctional AlkJ alcohol dehydrogenases (E_{di}) and bifunctional alcohol dehydrogenases (E_{dii}) of EC 1.1.1.1 or EC 1.1.1.2, wherein E_c, E_{di}, and E_{dii} are capable of oxidizing an 1-alkanol via an 1-alkanal directly to the corresponding alkanoic acid;
 - 25 - the Enzyme E₄ is selected from the group consisting of wax-ester synthases (E_f) and alcohol O-acyl transferases (E_g);
 - the Enzyme E₅ is selected from the group consisting of P450 alkane hydroxylases (E_a) and AlkB alkane hydroxylases (E_b) of EC 1.14.15.3;
 - and when present the Enzyme E₆ is selected from the group consisting of P450
 - 30 alkane hydroxylases (E_a), AlkB alkane hydroxylases (E_b) of EC 1.14.15.3, alcohol oxidases (E_c) and alcohol dehydrogenases (E_d);
 - when present the Enzyme E₇ is an ω -transaminase (E_h);
 - when present the Enzyme E₁₃ is selected from the group consisting of P450
 - 35 alkane hydroxylases (E_a) of EC 1.14.15.3-, AlkB alkane hydroxylases (E_b) of EC 1.14.15.3, aldehyde dehydrogenases (E_e), bifunctional alcohol oxidases (E_c) of EC 1.1.3.20, bifunctional AlkJ alcohol dehydrogenases (E_{di}) and bifunctional alcohol dehydrogenases (E_{dii}) of EC 1.1.1.1 or EC 1.1.1.2, wherein E_c, E_{di}, and E_{dii} are capable of oxidizing an ω -hydroxy alkanoic acid ester via an ω -oxo

alkanoic acid ester directly to the corresponding ω -carboxy alkanoic acid ester;
and

- when present the Enzyme E₁₄ is selected from the group consisting of wax-ester synthases (E_f) and alcohol O-acyl transferases (E_g).

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12. The method according to any one of claims 9 to 11, wherein the Enzymes E₁, E₂, E₃, and E₅ are at least one AlkB alkane hydroxylase (E_b) and when present E₆ is at least one AlkB alkane hydroxylase (E_b) and Enzyme E₄ is a wax-ester synthase (E_f).

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13. The method according to any one of claims 9 to 12, wherein the enzymes are brought into contact with the alkane in the form of a cell genetically modified to increase the expression relative to a wild type cell of enzymes E₁ to E₅ and optionally E₆ and E₇.

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14. The method according to claim 13, wherein the cell is selected from the group consisting of *E. coli*, *Pseudomonas* sp., *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas stutzeri*, *Acinetobacter* sp., *Burkholderia* sp., *Burkholderia thailandensis*, *Cyanobakterien*, *Klebsiella* sp., *Klebsiella oxytoca*, *Salmonella* sp., *Rhizobium* sp. and *Rhizobium meliloti*, *Bacillus* sp., *Bacillus subtilis*, *Clostridium* sp., *Corynebacterium* sp., *Corynebacterium glutamicum*, *Brevibacterium* sp., *Chlorella* sp. and *Nostoc* sp..

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15. The method according to any one of claims 9 to 14, wherein

- the ω -functionalized carboxylic acid ester is 12-amino lauric acid methyl ester, 12-hydroxy lauric acid methyl ester, 12-carboxy lauric acid methyl (di) ester and/or lauric acid methyl ester from the alkane dodecane; and/or
- the ω -functionalized carboxylic acid ester is 11-amino undecanoic acid methyl ester, 11-hydroxy undecanoic acid methyl ester, 11-carboxy undecanoic acid methyl (di) ester and/or undecanoic acid methyl ester from the alkane undecane.

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