

ω -AMINO CARBOXYLIC ACIDS, ω -AMINO CARBOXYLIC ACID ESTERS, OR
RECOMBINANT CELLS WHICH PRODUCE LACTAMS THEREOF

The present invention relates to cells that are genetically modified relative to their wild type, a method for the production of a genetically modified cell, the cells obtainable by this method, a method for the production of ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or of lactams derived from ω -aminocarboxylic acids, the ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids obtainable by this method, a method for the production of polyamides based on ω -aminocarboxylic acids or on lactams and the polyamides obtainable by this method.

Polyamides are polymers whose repeating units (monomers) possess the amide group as a characteristic feature. The designation "polyamides" is usually used to designate synthetic, commercially usable thermoplastics and therefore demarcates this class of substances from the chemically related proteins. Nearly all the important polyamides are derived from primary amines, i.e. the functional group $-\text{CO}-\text{NH}-$ occurs in their repeat units. Polyamides of secondary amines ($-\text{CO}-\text{NR}-$, R = organic residue) also exist. Aminocarboxylic acids, lactams and/or diamines and dicarboxylic acids in particular find application as monomers for the polyamides.

The production of polyamides on the basis of lactams is particularly important. Thus, "polyamide 6", a product that is widely used in industry, is obtained by ring opening polymerization of ϵ -caprolactam, whereas "polyamide 12", which is also industrially important, is obtained by ring opening polymerization of laurinelactam. Copolymers of lactams, such as copolymers of ϵ -caprolactam and laurinelactam ("polyamide 6/12") are also of considerable commercial importance.

The production of ϵ -caprolactam is usually carried out by reacting cyclohexanone with the hydrogensulphate or the hydrochloride of hydroxylamine with formation of cyclohexanone oxime. This is converted by a Beckmann rearrangement into ϵ -caprolactam, often with the use of concentrated sulphuric acid as catalyst. Cyclohexanone is usually produced by catalytic

oxidation of cyclohexane with oxygen of the air, cyclohexane being obtained in its turn by hydrogenation of benzene.

The production of laurinelactam is particularly expensive. On an industrial scale this first involves the trimerization of butadiene, with formation of cyclododecatriene. The cyclododecatriene is then hydrogenated with formation of cyclododecane and the cyclododecane obtained is oxidized with formation of cyclododecanone. The cyclododecanone thus obtained is then reacted with hydroxylamine to cyclododecane oxime, which is then converted in a Beckmann rearrangement to laurinelactam.

The disadvantage of these methods known from the prior art for the production of lactams by Beckmann rearrangement of oximes is, among other things, that large amounts of salts, for example sodium sulphate, are formed as by-product, which requires disposal. Therefore other methods for the production of lactams are also described in the prior art, which do not have these disadvantages. Thus, EP-A-0 748 797 describes a method for the production of lactams from dinitriles, in which the dinitrile is hydrogenated to aminonitrile and the aminonitrile is converted by cyclizing hydrolysis to the lactam. Molecular sieves, such as acid zeolites, silicates and non-zeolitic molecular sieves, metal phosphates and metal oxides or mixed metal oxides have been disclosed as catalyst for cyclizing hydrolysis. However, this method has, among other drawbacks, the disadvantage that the selectivity of the conversion of the aminonitrile by cyclizing hydrolysis is rather low and therefore large amounts of by-products are formed. Furthermore, in the methods for the production of lactams described from this prior art, hydrocarbons such as benzene or butadiene are used, which are obtained by cracking gasoline or petroleum and therefore are not derived from renewable raw materials. The production of polyamides, which are based on lactams produced in this way, is therefore to be regarded as disadvantageous from the environmental standpoint.

The present invention was based on the aim of overcoming the disadvantages arising from the prior art.

In particular the present invention was based on the aim of providing a method by which lactams, in particular laurinolactam, can be formed in the fewest possible steps and with formation of the minimum possible amount of by-products.

Another aim of the present compound was to provide a method by which lactams, in particular laurinolactam, can be produced from renewable raw materials.

A contribution to achievement of the aforementioned aims is provided by a cell, which has been genetically modified relative to its wild type so that, in comparison with its wild type, it is able to produce more ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or more lactams derived from ω -aminocarboxylic acids, starting from carboxylic acids or carboxylic acid esters. Such a cell can be used in order to produce ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids by fermentation from carboxylic acids or carboxylic acid esters, for example from lauric acid or lauric acid esters.

The formulation "*that in comparison with its wild type it is able to produce more ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or more lactams derived from ω -aminocarboxylic acids, starting from carboxylic acids or carboxylic acid esters*" also applies to the case when the wild type of the genetically modified cell is not able to form any ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or any lactams derived from ω -aminocarboxylic acids, or at least no detectable amounts of these compounds and it is only after the genetic modification that detectable amounts of these components can be formed.

A "*wild type*" of a cell preferably denotes a cell whose genome is in a state such as arose naturally by evolution. The term is used both for the whole cell and for individual genes. The term "*wild type*" therefore in particular does not include such cells or such genes whose gene sequences have been altered at least partially by man by recombinant methods.

It is preferable according to the invention for the genetically modified cell to have been genetically modified so that in a defined time interval, preferably within 2 hours, still more preferably within 8 hours and most preferably within 24 hours, it forms at least twice, especially

preferably at least 10 times, even more preferably at least 100 times, and yet more preferably at least 1000 times and most preferably at least 10000 times more ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids than the wild-type cell. The increase in product formation can be determined for example by cultivating the cell according to the 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 -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids) in the nutrient medium.

The cells according to the invention can be prokaryotes or eukaryotes. They can be mammalian cells (such as human cells), plant cells or microorganisms such as yeasts, fungi or bacteria, microorganisms being especially preferred and bacteria and yeasts being most preferred.

Suitable bacteria, yeasts or fungi are in particular those bacteria, yeasts or fungi that have been deposited in the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, abbreviated to DSMZ), Brunswick, Germany, as strains of bacteria, yeasts or fungi. Suitable bacteria according to the invention belong to the genera listed at

<http://www.dsmz.de/species/bacteria.htm>

suitable yeasts according to the invention belong to the genera listed at

<http://www.dsmz.de/species/yeasts.htm>

and suitable fungi according to the invention are those listed at

<http://www.dsmz.de/species/fungi.htm>

Cells that are especially preferred according to the invention are derived from cells of the genera *Corynebacterium*, *Brevibacterium*, *Bacillus*, *Lactobacillus*, *Lactococcus*, *Candida*, *Pichia*, *Kluveromyces*, *Saccharomyces*, *Escherichia*, *Zymomonas*, *Yarrowia*, *Methylobacterium*, *Ralstonia*, *Pseudomonas*, *Burkholderia* and *Clostridium*, with *Escherichia coli*, *Corynebacterium glutamicum* and *Pseudomonas putida* being especially preferred and *Escherichia coli* being most preferred.

According to a preferred embodiment of the cell according to the invention the latter displays, in comparison with its wild type, increased activity of at least one of the following enzymes:

- i) an enzyme E_I, which catalyses the conversion of carboxylic acids or carboxylic acid esters to the corresponding ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters;
- ii) an enzyme E_{II}, which catalyses the conversion of ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters to the corresponding ω -oxocarboxylic acids or ω -oxocarboxylic acid esters;
- iii) an enzyme E_{III}, which catalyses the conversion of ω -oxocarboxylic acids or ω -oxocarboxylic acid esters to the corresponding ω -aminocarboxylic acids or ω -aminocarboxylic acid esters.

The term "*increased activity of an enzyme*", as used above in connection with the enzyme E_I and hereinafter in connection with the enzymes E_{II} etc., is preferably to be understood as increased intracellular activity.

The following account regarding the increase in enzyme activity in cells applies both to the increase in activity of the enzyme E_I and to all the enzymes stated subsequently, whose activity can possibly be increased.

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 and optionally by combining these measures. Genetically modified cells according to the 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 extrachromosomally replicating vector.

A review of possible ways of increasing the enzyme activity in cells for the example of pyruvate carboxylase is given in DE-A-100 31 999, which is hereby incorporated as reference and whose disclosures with respect to the possibilities for increasing the enzyme activity in cells forms part of the disclosure of the present invention.

The expression of the aforementioned and all subsequently mentioned enzymes or genes can be detected by means of 1- and 2-dimensional protein gel separation and subsequent optical identification of the protein concentration in the gel using appropriate evaluation software. If the increase in enzyme activity is based exclusively on an increase in expression of the corresponding gene, the increase in enzyme activity can be quantified in a simple way by comparing the 1- or 2-dimensional protein separations between wild type and genetically modified cell. A usual method for the preparation of protein gels in the case of coryneform 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 that is specific for the protein that is to be detected (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 determination of concentration (Lohaus and Meyer (1989) *Biospektrum*, 5: 32-39; Lottspeich (1999), *Angewandte Chemie* 111: 2630-2647). The activity of DNA-binding proteins can be measured by DNA-Band-Shift-Assays (also called gel retardation) (Wilson et al. (2001) *Journal of Bacteriology*, 183: 2151-2155). The action of DNA-binding proteins on the expression of other genes can be detected by various well-described methods of reporter gene assay (Sambrook et al., *Molecular Cloning: a laboratory manual*, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. USA, 1989). Intracellular enzymatic activities can be determined by various methods that have been described (Donahue et al. (2000) *Journal of*

Bacteriology 182 (19): 5624-5627; Ray et al. (2000) *Journal of Bacteriology* 182 (8): 2277-2284; Freedberg et al. (1973) *Journal of Bacteriology* 115 (3): 816-823). If in the subsequent account no concrete methods are stated for determination of the activity of a particular enzyme, the increase in enzyme activity as well as the decrease in enzyme activity are preferably determined by the methods described in Hermann et al., *Electrophoresis*, 22: 1712-23 (2001), Lohaus et al., *Biospektrum* 5 32-39 (1998), Lottspeich, *Angewandte Chemie* 111: 2630-2647 (1999) and Wilson et al., *Journal of Bacteriology* 183: 2151-2155 (2001).

If the increase in enzyme activity is brought about by mutation of the endogenous gene, such mutations can either be produced undirected according to classical methods, such as by UV-irradiation or by mutation-causing chemicals, or purposefully by genetic engineering methods such as deletion(s), insertion(s) and/or nucleotide exchange(s). Genetically modified cells are obtained as a result of these mutations. Especially preferred mutants of enzymes are in particular also enzymes for which feedback inhibition is no longer present or at least is reduced in comparison with the wild-type enzyme.

If the increase in enzyme activity is brought about through an increase in expression of an enzyme, then for example we increase the copy number of the corresponding genes or mutate the promoter and regulating region or the ribosome binding site, which is located upstream of the structural gene. Expression cassettes that are inserted upstream of the structural gene work in this way. By means of inducible promoters it is additionally possible to increase the expression at any time. Moreover, the enzyme gene can also be assigned, as regulatory sequences, so-called "enhancers", which as a result of improved interaction between RNA-polymerase and DNA also bring about increased gene expression. Expression is also improved by measures for extending the life of the m-RNA. Furthermore, by preventing the degradation of the enzyme protein, enzyme activity is also intensified. The genes or gene constructs are then either contained in plasmids with varying copy number or are integrated in the chromosome and amplified. Alternatively, overexpression of the relevant genes can in addition be achieved by altering the composition of the medium and the culture conditions. A person skilled in the art will find instructions for this in, *inter alia*, Martin et al. (*Bio/technology* 5, 137-146 (1987)), Guerrero et al. (*Gene* 138, 35-41 (1994)), Tsuchiya and Morinaga (*Bio/technology* 6, 428-430 (1988)),

Eikmanns et al. (*Gene* 102, 93-98 (1991)), in EP-A-0 472 869, in US 4,601,893, in Schwarzer and Pühler (*Bio/technology* 9, 84-87 (1991), in Reinscheid et al. (*Applied and Environmental Microbiology* 60, 126-132 (1994)), in LaBarre et al. (*Journal of Bacteriology* 175, 1001-1007 (1993)), in WO-A-96/15246, in Malumbres et al. (*Gene* 134, 15-24 (1993), in JP-A-10-229891, in Jensen and Hammer (*Biotechnology and Bioengineering* 58, 191-195 (1998)) and in known textbooks of genetics and molecular biology. The measures described above also lead, like mutations, to genetically modified cells.

Episomal plasmids, for example, are used for increasing the expression of the genes in question. Suitable plasmids are in particular those that are replicated in coryneform bacteria. Numerous known plasmid vectors, for example pZ1 (Menkel et al., *Applied and Environmental Microbiology* 64: 549-554 (1989)), pEKEx1 (Eikmanns et al., *Gene* 107: 69-74 (1991)) or pHS2-1 (Sonnen et al., *Gene* 107: 69-74 (1991)) are based on the cryptic plasmids pHM1519, pBL1 or pGA1. Other plasmid vectors, for example those based on pCG4 (US 4,489,160) or pNG2 (Serwold-Davis et al., *FEMS Microbiology Letters* 66: 119-124 (1990)) or pAG1 (US 5,158,891), can be used in the same way.

Furthermore, plasmid vectors are also suitable, by means of which we can apply the method of gene amplification by integration into the chromosome, as was described for example by Reinscheid et al. (*Applied and Environmental Microbiology* 60: 126-132 (1994)) for the duplication or amplification of the *hom-thrB* operon. In this method the complete gene is cloned into a plasmid vector, which can be replicated in a host (typically *Escherichia coli*), but not in *Corynebacterium glutamicum*. Vectors that may be considered are for example pSUP301 (Simon et al., *Bio/Technology* 1: 784-791 (1983)), pK18mob or pK19mob (Schäfer et al., *Gene* 145: 69-73 (1994)), pGEM-T (Promega Corporation, Madison, Wisconsin, USA), pCR2.1-TOPO (Shuman, *Journal of Biological Chemistry* 269: 32678-84 (1994)), pCR[®]Blunt (Invitrogen, Groningen, The Netherlands), pEM1 (Schrumpf et al., *Journal of Bacteriology* 173: 4510-4516)) or pBGS8 (Spratt et al., *Gene* 41: 337-342 (1986)). The plasmid vector that contains the gene to be amplified is then transferred by conjugation or transformation into the desired strain of *Corynebacterium glutamicum*. The method of conjugation is described for example in Schäfer et al., *Applied and Environmental Microbiology* 60: 756-759 (1994). Methods for transformation

are described for example in Thierbach et al., *Applied Microbiology and Biotechnology* 29: 356-362 (1988), Dunican and Shivnan, *Bio/Technology* 7: 1067-1070 (1989) and Tauch et al., *FEMS Microbiology Letters* 123: 343-347 (1994). After homologous recombination by a "cross-over" event, the resultant strain contains at least two copies of the relevant gene.

The formulation used in the above and hereinafter "*increased activity of an enzyme E_x relative to its wild type*" preferably always means activity of the respective enzyme E_x that is increased by a factor of at least 2, especially preferably of at least 10, even more preferably of at least 100, yet more preferably of at least 1000 and most preferably of at least 10000. Furthermore, the cell according to the invention, which has "*increased activity of an enzyme E_x relative to its wild type*", in particular also comprises a cell whose wild type has no or at least no detectable activity of this enzyme E_x and only displayed a detectable activity of this enzyme E_x after the enzyme activity was increased, for example through overexpression. In this connection the term "*overexpression*" or the formulation "*increase in expression*" used hereinafter also includes the case when a starting cell, for example a wild-type cell, has no or at least no detectable expression and it is only by recombinant methods that a detectable expression of the enzyme E_x is induced.

Furthermore, according to the invention it is preferable for the cell to have increased activity of enzymes E_I and E_{II} , enzymes E_I and E_{III} , enzymes E_{II} and E_{III} or even increased activity of all the enzymes E_I , E_{II} and E_{III} .

Furthermore, in connection with the aforementioned preferred embodiment of the cell according to the invention it is preferable for

- enzyme E_I to be an alkane monooxygenase or a xylene monooxygenase, or, preferably and
- enzyme E_{II} to be an alkane monooxygenase, an alcohol dehydrogenase or an alcohol oxidase, or, preferably and
- enzyme E_{III} to be a ω -transaminase.

A preferred enzyme E_I , in particular a preferred alkane monooxygenase is the alkane monooxygenase encoded by the *alkBGT* gene from *Pseudomonas putida* GPO1. The isolation of

the *alkBGT* gene sequence is described for example by van Beilen et al. in "*Functional Analysis of Alkane Hydroxylases from Gram-Negative and Gram-Positive Bacteria*", Journal of Bacteriology, Vol. 184 (6), pages 1733-1742 (2002). Furthermore, cytochrome P450 monooxygenases, in particular cytochrome P450 monooxygenases from *Candida*, for example from *Candida tropicalis*, or from plants, for example from the chick-pea (*Cicer arietinum L.*), can also be used as alkane monooxygenases. The gene sequences of suitable cytochrome P450 monooxygenases from *Candida tropicalis* are for example disclosed in WO-A-00/20566, whereas the gene sequences of suitable cytochrome P450 monooxygenases from the chick-pea are given for example by Barz et al. in "*Cloning and characterization of eight cytochrome P450 cDNAs from chickpea (Cicer arietinum L.) cell suspension cultures*", Plant Science, Vol. 155, pages 101-108 (2000). Other homologues of the *alkB* gene are also given by van Beilen et al. in "*Oil & Gas Science and Technology*", Vol. 58 (4), pages 427-440 (2003). A suitable gene for a xylene monooxygenase is for example the *xylM* or the *xylA* gene, and a plasmid containing these two genes has the GENBANK Accession No. M37480.

A preferred enzyme E_{II}, in particular a preferred alcohol dehydrogenase is for example the alcohol dehydrogenase encoded by the *alkJ* gene (EC 1.1.99-2), in particular the alcohol dehydrogenase encoded by the *alkJ* gene from *Pseudomonas putida* GPo1. The gene sequences the alcohol dehydrogenase encoded by the *alkJ* gene from *Pseudomonas putida* GPo1, *Alcanivorax borkumensis*, *Bordetella parapertussis*, *Bordetella bronchiseptica* or from *Roseobacter denitrificans* can be found for example in the KEGG gene databank.

Suitable ω -transaminases are for example the ω -transaminases that are characterized in US-A-2007/0092957 by the sequence numbers 248, 250, 252 and 254.

A preferred enzyme E_{III}, in particular a preferred ω -transaminase is in particular the ω -transaminase from *Chromobacterium violaceum* DSM30191 (Kaulmann et al., 2007; "*Substrate spectrum of ω -transaminase from Chromobacterium violaceum DSM30191 and its potential for biocatalysis*", Enzyme and Microbial Technology, Vol. 41, pages 628-637), which is encoded by the gene sequence according to SEQ ID No. 01.

It can be advantageous to use, as enzyme E_{III}, ω -transaminases that can be isolated from plants. The ω -transaminases from plants selected from the group comprising *Arabidopsis thaliana*, *Avena sativa*, *Beta vulgaris*, *Glycine max*, *Hordeum vulgare*, *Lotus japonicus*, *Solanum lycopersicum*, *Manihot esculenta*, *Oryza sativa*, *Triticum aestivum*, *Zea mays*, *Spinacia oleracea*, *Arum maculatum*, *Mercurialis perennis* and *Urtica dioica*, are preferred here, and *Arabidopsis thaliana* is especially preferred. Enzymes that are encoded by nucleic acids that have 90%, preferably 95%, especially preferably 99 and quite especially preferably 100% identity to the sequence according to SEQ ID No. 39, are suitable in particular as ω -transaminases. The "nucleotide identity" relative to SEQ ID No. 39 is determined using known methods. In general, special computer programs with algorithms are used, taking into account special requirements. Preferred methods for determination of identity first produce the greatest agreement between the sequences to be compared. Computer programs for determination of identity comprise, but are not restricted to, the GCG software package, including

- GAP (Deveroy, J. et al., Nucleic Acid Research 12 (1984), page 387, Genetics Computer Group University of Wisconsin, Medicine (WI), and
- BLASTP, BLASTN and FASTA (Altschul, S. et al., Journal of Molecular Biology 215 (1990), pages 403-410. The BLAST program can be obtained from the National Center for Biotechnology Information (NCBI) and from other sources (BLAST Manual, Altschul S. et al., NCBI NLM NIH Bethesda ND 22894; Altschul S. et al., as above).

The well-known Smith-Waterman algorithm can also be used for determining nucleotide identity.

Preferred parameters for nucleotide comparison comprise the following:

- Algorithm Needleman and Wunsch, Journal of Molecular Biology 48 (1970), pages 443-453
 - Comparison matrix
- | | | |
|--------------------|---|------|
| Matches | = | + 10 |
| Mismatches | = | 0 |
| Gap penalty | = | 50 |
| Gap length penalty | = | 3 |

The GAP program is also suitable for use with the parameters given above. The aforementioned parameters are the default parameters in the nucleotide sequence comparison.

Moreover, enzymes from the subgroup of the β -Ala:pyruvate transaminases are suitable. These include e.g. transaminases from *Pseudomonas putida* W619 (gi: 119860707, gi: 119855857, gi: 119856278), from *Pseudomonas putida* KT2440 (gi: 24984391), from *Pseudomonas aeruginosa* PA01 (gi: 15595330, gi: 15600506, gi: 15595418, gi: 9951072); *Streptomyces coelicolor* A3(2) (gi: 3319731), *Streptomyces avermitilis* MA 4680 (gi: 29831094, gi: 29829154) and *Chromobacterium violaceum* ATCC 12472 (gi: 34102747). The amino acid sequences of the aforementioned transaminases are presented in the sequences according to SEQ ID No. 19 to SEQ ID No. 30.

For the case when the cells according to the invention are to be used for the production of ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams based on ω -aminocarboxylic acids starting from carboxylic acid esters, it is moreover advantageous if the cell according to the invention has, in addition to increased activity of at least one of the enzymes E_I , E_{II} and E_{III} , preferably in addition to increased activity of the enzymes E_I and E_{III} or E_I , E_{II} and E_{III} , also increased activity of an enzyme E_{IV} , which catalyses the conversion of ω -aminocarboxylic acid esters to the corresponding ω -aminocarboxylic acids, said enzyme E_{IV} preferably being an esterase, which preferably is secreted by the cell. Secretion of the esterase by the cell has the advantage that the ester bond is only cleaved outside of the cell. This ensures that, owing to the better membrane permeability of the ω -aminocarboxylic acid ester compared with ω -aminocarboxylic acid, sufficient target product leaves the cell and can be transferred to the nutrient medium surrounding the cell.

Preferred esterases according to the invention are in particular lipase, and as an example of a suitable lipase we may mention the lipase LipA from *Pseudomonas fluorescens* HU380 (ACC Code Q76D26, Kojima and Shimizu, "Purification and Characterization of the Lipase from *Pseudomonas fluorescens* HU380", *Journal of Bioscience and Bioengineering*, Volume 96 (3), pages 219-226 (2003)). In order to ensure that the esterases are secreted, they can be provided, in a manner known by a person skilled in the art, with corresponding signal sequences, which establish secretion. If for example the aforementioned lipase LipA from *Pseudomonas fluorescens* HU380 is overexpressed in *E. coli*, it can be provided advantageously with signal

sequences from EstA, an esterase that occurs naturally on the cell surface of *Pseudomonas aeruginosa* (Becker et al., "A generic system for the *Escherichia coli* cell-surface display of lipolytic enzymes", *FEBS Letters*, Vol. 579, pages 1177-1182 (2005)). Other suitable enzymes are lipases from *C. antarctica*, *M. miehei* and *P. cepacia* (Vaysse et al., "Enzyme and Microbial Technology", Vol. 31, pages 648-655 (2002)).

Alternatively the secreted ω -aminocarboxylic acid ester can also be cleaved conventionally, to obtain the ω -aminocarboxylic acid, for example by saponification, i.e. hydrolysis of the ω -aminocarboxylic acid ester by the aqueous solution of a hydroxide, e.g. by sodium hydroxide.

Furthermore, it may prove advantageous according to the invention if the cell according to the invention, in addition to increased activity of at least one of the enzymes E_I , E_{II} and E_{III} , preferably in addition to increased activity of the enzymes E_I and E_{III} or E_I , E_{II} and E_{III} and optionally also in addition to increased activity of the aforementioned enzyme E_{IV} , also has increased activity of an enzyme E_V , which catalyses the conversion of ω -aminocarboxylic acids to the corresponding lactams, and it can also be advantageous here if this enzyme E_V is secreted by the cell. In this way it can be possible for the ω -aminocarboxylic acids formed directly by the cell or the ω -aminocarboxylic acid that is only formed after extracellular cleavage of ω -aminocarboxylic acid esters to be converted to the corresponding lactam, thus optionally facilitating purification of the target product.

According to another, special embodiment of the cell according to the invention, it has, in addition to increased activity of one or more of the enzymes E_I , E_{II} or E_{III} and optionally increased activity of the enzyme E_{IV} and/or E_V , also increased activity of an enzyme E_{VI} , which catalyses the conversion of an α -ketocarboxylic acid to an amino acid, said enzyme E_{VI} preferably being an amino acid dehydrogenase. Such a modification of the cell would have the advantage that in the case when amino acids are used as donor for the NH_2 group, which is consumed during the transaminase (E_{III})–mediated reaction of an ω -oxocarboxylic acid or an ω -oxocarboxylic acid ester to the corresponding ω -aminocarboxylic acid, to the corresponding ω -aminocarboxylic acid ester or to the corresponding ω -aminocarboxylic acid ester, can be correspondingly regenerated. Preferred, as amino acid dehydrogenase, is the alanine dehydrogenase from *B. subtilis* (EC No. 1.4.1.1; Gene ID: 936557), which is encoded by the

gene sequence according to SEQ ID No. 02. Other suitable amino acid dehydrogenases are serine dehydrogenases, aspartate dehydrogenases, phenylalanine dehydrogenases and glutamate dehydrogenases.

A contribution to achievement of the aims stated at the beginning is also provided by a method for the production of a genetically modified cell, comprising the process step of increasing the activity of at least one of the following enzymes:

- i) an enzyme E_I, which catalyses the conversion of carboxylic acids or carboxylic acid esters to the corresponding ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters,
- ii) an enzyme E_{II}, which catalyses the conversion of ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters to the corresponding ω -oxocarboxylic acids or ω -oxocarboxylic acid esters, or
- iii) an enzyme E_{III}, which catalyses the conversion of ω -oxocarboxylic acids or ω -oxocarboxylic acid esters to the corresponding ω -aminocarboxylic acids or ω -aminocarboxylic acid esters,

in a cell, with the enzyme activities preferably being increased by the methods described at the beginning.

According to a special embodiment of the method described above, in this method, in addition to the increase in activity of the enzymes E_I, E_{II} and/or E_{III}, the activity of an enzyme E_{IV}, which catalyses the conversion of ω -aminocarboxylic acid esters to the corresponding ω -aminocarboxylic acids, and/or of an enzyme E_V, which catalyses the conversion of ω -aminocarboxylic acids to the corresponding lactams, is also increased by increasing the expression of these enzymes, with the enzymes E_{IV} and/or E_V preferably being secreted by the cell.

A contribution to achievement of the aims stated at the beginning is also provided by the genetically modified cells that are obtainable by the method described above.

Another contribution to achievement of the cells stated at the beginning is provided by a method for the production of ω -aminocarboxylic acids, of ω -aminocarboxylic acid esters or of lactams derived from ω -aminocarboxylic acids, containing the process steps:

- I) contacting a cell according to the invention with a culture medium containing a carboxylic acid or a carboxylic acid ester or with a culture medium contiguous with an organic phase containing a carboxylic acid or a carboxylic acid ester in conditions that enable the cell to form ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids, from the carboxylic acid or from the carboxylic acid esters;
- II) optionally isolation of the resultant ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids.

In step I) of the method according to the invention the cells are first brought into contact with a culture medium containing a carboxylic acid or a carboxylic acid ester or with a culture medium contiguous with an organic phase containing a carboxylic acid or a carboxylic acid ester, and this contacting takes place under conditions that make it possible for the cell to form ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids, from the carboxylic acid or from the carboxylic acid esters.

The genetically modified cells according to the invention can be brought into contact with the nutrient medium, and therefore cultivated continuously or discontinuously in a *batch process* or in a *fed-batch process* or in a *repeated-fed-batch process*, for the purpose of producing ω -aminocarboxylic acids or lactams derived from ω -aminocarboxylic acids. A semi-continuous process is also conceivable, as described in GB-A-1009370. Known culture methods are described in Chmiel's textbook ("*Bioprozesstechnik 1. Einführung in die Bioverfahrenstechnik*" [Bioprocess Techniques 1. Introduction to Bioprocess Engineering] (Gustav Fischer Verlag, Stuttgart, 1991)) or in the textbook by Storhas ("*Bioreaktoren und periphere einrichtungen*" [Bioreactors and Peripheral Equipment], Vieweg Verlag, Brunswick/Wiesbaden, 1994).

The culture medium to be used must be suitable for the requirements of the particular strains. Descriptions of culture media for various microorganisms are given in "*Manual of Methods for*

General Bacteriology" of the American Society for Bacteriology (Washington D.C., USA, 1981).

Apart from the carboxylic acids or carboxylic acid esters, the carbon source used can be carbohydrates, e.g. glucose, sucrose, lactose, fructose, maltose, molasses, starch and cellulose, oils and fats, e.g. soya oil, sunflower oil, peanut oil and coconut oil, fatty acids e.g. palmitic acid, stearic acid and linoleic acid, alcohols e.g. glycerol and methanol, hydrocarbons such as methane, amino acids such as L-glutamate or L-valine or organic acids e.g. acetic acid. These substances can be used separately or as a mixture. The use of carbohydrates, especially monosaccharides, oligosaccharides or polysaccharides, is especially preferred, as described in US 6.01.494 and US 6,136,576, and of C₅-sugars or glycerol.

Organic nitrogen-containing compounds such as peptones, yeast extract, meat extract, malt extract, corn-steep liquor, soybean flour and urea or inorganic compounds such as ammonium sulphate, ammonium chloride, ammonium phosphate, ammonium carbonate and ammonium nitrate can be used as the nitrogen source. The nitrogen sources can be used separately or as a mixture.

Phosphoric acid, potassium dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium-containing salts can be used as the source of phosphorus. The culture medium must in addition contain salts of metals, for example magnesium sulphate or iron sulphate, which are required for growth. Finally, essential growth substances such as amino acids and vitamins are used in addition to the substances mentioned above. Furthermore, suitable precursors can be added to the culture medium. The stated substances can be added to the culture in the form of a single preparation, or they can be supplied in a suitable manner during cultivation.

Basic compounds such as sodium hydroxide, potassium hydroxide, ammonia or ammonia water or acid compounds such as phosphoric acid or sulphuric acid are used in a suitable manner for controlling the pH of the culture. Antifoaming agents, e.g. fatty acid polyglycol esters, are used for controlling foaming. To maintain plasmid stability, suitable selectively acting substances, e.g.

antibiotics, can be added to the medium. To maintain aerobic conditions, oxygen or oxygen-containing gas mixtures, e.g. air, are fed into the culture. The temperature of the culture is normally in the range from 20°C to 45°C and preferably 25°C to 40°C.

According to an especially preferred embodiment of the method according to the invention for the production of ω -aminocarboxylic acids, of ω -aminocarboxylic acid esters or of lactams derived from ω -aminocarboxylic acids, in which a recombinant cell, derived from an *E. coli* cell, is used as the cell according to the invention, a mineral salt medium supplemented with ampicillin, chloramphenicol and kanamycin according to Riesenberget al., "*High cell density fermentation of recombinant Escherichia coli expressing human interferon alpha 1*", *Appl Microbiol and Biotechnol*gy, Vol. 34 (1), pages 77-82 (1990)) is used as the nutrient medium.

The contacting of the cells according to the invention with the culture medium in step I) preferably takes place in conditions that enable the cell to form ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids starting from carboxylic acid or from carboxylic acid esters. As carboxylic acids or carboxylic acid esters, consideration may be given in particular to carboxylic acids with number of carbons in the range from 6 to 20, especially preferably from 6 to 15, in particular from 6 to 12, lauric acid being especially preferred as carboxylic acid. As carboxylic acid esters, consideration may be given in particular to the methyl or ethyl esters of the aforementioned carboxylic acids, with the methyl ester of lauric acid being especially preferred as carboxylic acid ester.

In the production of the ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids, various procedures are conceivable in step I).

According to one embodiment of the method according to the invention, the cells are first cultivated, for the purpose of biomass production, in a nutrient medium that does not contain carboxylic acids or carboxylic acid esters, and in particular does not contain the aforementioned, preferred carboxylic acids or carboxylic acid esters. It is only after a certain biomass has been obtained that the carboxylic acids or the carboxylic acid esters are added to the nutrient medium or the cells are brought into contact with a new nutrient medium containing the carboxylic acids

or carboxylic acid esters. In this connection it is in particular preferable for the content of carboxylic acids or carboxylic acid esters during the formation of ω -aminocarboxylic acids, of ω -aminocarboxylic acid esters or of lactams derived from ω -aminocarboxylic acids to be in the range from 1 to 200 g/l, especially preferably in the range from 20 to 200 g/l.

According to another embodiment of the method according to the invention, it is carried out in a two phase system, containing

- A) an aqueous phase, and
- B) an organic phase,

with the formation of the ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids by the cells in step I) taking place in the aqueous phase and with the resultant ω -aminocarboxylic acids, the resultant ω -aminocarboxylic acid esters or the resultant lactams derived from ω -aminocarboxylic acids accumulating in the organic phase. In this way it is possible for the resultant ω -aminocarboxylic acids, the resultant ω -aminocarboxylic acid esters or the resultant lactams derived from ω -aminocarboxylic acids to be extracted *in situ*.

Also in this embodiment of the method according to the invention, it may prove advantageous for the cells first to be cultivated, for the purpose of biomass production, in a nutrient medium that does not contain carboxylic acids or carboxylic acid esters, and in particular does not contain the aforementioned, preferred carboxylic acids or carboxylic acid esters. It is only after a certain biomass has been obtained that the cell suspension as aqueous phase A) is brought into contact with the organic phase B), where in particular the organic phase B) contains the carboxylic acid or the carboxylic acid esters preferably in an amount in the range from 1 to 200 g/l, especially preferably in the range from 20 to 200 g/l. However, if substrates that are not toxic to the cells used, such as methyl laurate, are employed as carboxylic acids or carboxylic acid esters, then the content of these carboxylic acids or carboxylic acid esters in the organic phase can also be significantly higher. In such a case it may also be possible to use the pure carboxylic acid or the pure carboxylic acid esters, for example pure methyl laurate, as organic phase.

As organic phase, it is possible to use alkanes of medium chain length, preferably those with a logP value of more than 4 (little foam formation), or physically similar aromatics or aromatic esters, though preferably, as mentioned above, lauric acid esters, especially preferably methyl laurate, BEHP (bis(2-ethylhexyl)phthalate) or long-chain fatty acid esters (biodiesel).

Furthermore it is preferable according to the invention if, at least during the phase of formation of ω -aminocarboxylic acids, of ω -aminocarboxylic acid esters or of lactams derived from ω -aminocarboxylic acids, the culture medium used in step I) contains amino group donors, such as ammonia or ammonium ions or even amino acids, though in particular alanine or aspartate, which function as amine donors in the transaminase-catalysed conversion of the ω -oxocarboxylic acids or the ω -oxocarboxylic acid esters to the corresponding ω -aminocarboxylic acids or ω -aminocarboxylic acid esters.

In step II) of the method according to the invention, the resultant ω -aminocarboxylic acids, the resultant ω -aminocarboxylic acid esters or the lactams derived from the ω -aminocarboxylic acids are optionally isolated, and it is preferable for this isolation to take place in an at least two-stage purification process, comprising

- a) an extraction step, in which the ω -aminocarboxylic acids, the ω -aminocarboxylic acid esters or the lactams derived from ω -aminocarboxylic acids are extracted from the culture medium, and
- b) a fine purification step, in which the extract obtained in step a) is purified further by a distillation process or selective crystallization, obtaining an ω -aminocarboxylic acid phase, an ω -aminocarboxylic acid ester phase or a lactam phase with a purity of at least 99.8%.

The extraction in step a) can in particular be designed as so-called "*in situ*" extraction, in which steps I) and II) of the method according to the invention for the production of ω -aminocarboxylic acids, of ω -aminocarboxylic acid esters or of lactams derived from ω -aminocarboxylic acids are carried out simultaneously. This "*in situ*" extraction has already been described above.

The fine purification in step II) can for example take place by distillation or crystallization.

In a special embodiment of the method according to the invention for the production of ω -aminocarboxylic acids, of ω -aminocarboxylic acid esters or of lactams derived from ω -aminocarboxylic acids, the ω -aminocarboxylic acid esters formed in step I) are reacted in another process step by conventional chemical methods to ω -aminocarboxylic acids; a preferred, conventional chemical method is saponification, in which the ω -aminocarboxylic acid ester is reacted with an aqueous solution of a base, preferably a hydroxide, preferably sodium hydroxide, to the ω -aminocarboxylic acid.

Preferably this method is used for the production of ω -aminolauric acid from lauric acid esters, preferably methyl laurate;

A contribution to achievement of the aims stated at the beginning is also provided by ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or by lactams derived from ω -aminocarboxylic acids, which are obtainable by the method described above, the lactam preferably being laurinlactam, which is obtained if lauric acid or lauric acid esters are used as carboxylic acid or as carboxylic acid esters in step I) of the method according to the invention for the production of lactams derived from ω -aminocarboxylic acids, wherein the ω -aminocarboxylic acid is preferably ω -aminolauric acid and the ω -aminocarboxylic acid ester is preferably ω -aminomethyl laurate.

A contribution to achievement of the aims stated at the beginning is also provided by a method for the production of polyamides based on ω -aminocarboxylic acids, comprising the process steps:

- (α 1) production of ω -aminocarboxylic acids by one of the methods described above for the production of ω -aminocarboxylic acids, in particular by the method described above for the production of ω -aminolauric acid from lauric acid or lauric acid esters;
- (α 2) polymerization of the ω -aminocarboxylic acid, obtaining a polyamide.

In step ($\alpha 2$) of the method according to the invention for the production of polyamides based on ω -aminocarboxylic acids, the ω -aminocarboxylic acids obtained in step ($\alpha 1$), in particular the ω -aminolauric acids obtained in step ($\alpha 1$), are converted in a polymerization to a polyamide, and optionally mixtures of various ω -aminocarboxylic acids can also be used, for which at least one of the ω -aminocarboxylic acids, but optionally all ω -aminocarboxylic acids were produced by the method according to the invention for the production of ω -aminocarboxylic acids.

The production of the polyamides from the ω -aminocarboxylic acids can be carried out by well-known methods, as described for example in L. Notarbartolo, Ind. Plast. Mod. 10(1958)2, p. 44, JP 01-074224, JP 01-051433, JP63286428, JP58080324 or JP60179425.

A contribution to achievement of the aims stated at the beginning is also provided by a method for the production of polyamides based on lactams, comprising the process steps:

- ($\beta 1$) production of lactams by the method described above for the production of lactams derived from ω -aminocarboxylic acids, in particular by the method described above for the production of laurinelactam from lauric acid or lauric acid esters;
- ($\beta 2$) ring opening polymerization or polycondensation of the laurinelactam, obtaining a polyamide.

In step ($\beta 2$) of the method according to the invention for the production of polyamides based on lactams, the lactams obtained in step ($\beta 1$), in particular the laurinelactam obtained in step ($\beta 1$), are converted in a ring opening polymerization or by polycondensation to a polyamide, and optionally it is also possible to use mixtures of various lactams, for example mixtures of laurinelactam and ϵ -caprolactam, for which at least one of the lactams, but optionally all lactams were produced by the method according to the invention for the production of lactams derived from ω -aminocarboxylic acids.

The production of the polyamides from the lactams can be carried out by well-known methods, as described for example in DE-A-14 95 198, DE-A-25 58 480, EP-A-0 129 196 or also in

"*Polymerization Processes*", Interscience, New York, 1977, pages 424-467, especially pages 444-446.

A contribution to achievement of the aims stated at the beginning is also provided by polyamides, which are obtainable by the methods described above. It is especially preferable for these polyamides to be based, up to at least 10 wt.%, especially preferably up to at least 25 wt.%, still more preferably up to at least 50 wt.% and most preferably up to at least 75 wt.%, on lauric acid, lauric acid ester or laurinlactam, obtained by the method according to the invention for the production of lauric acid, of lauric acid ester or of laurinlactam from lauric acid or lauric acid esters.

The invention will now be explained with the aid of non-limiting diagrams and examples.

Fig. 1 shows, schematically, a recombinant plasmid for chromosomal integration of the *alk* genes in *Escherichia coli* (tnp: transposase gene; bla: ampicillin resistance gene; oriT RP4: mobilization region; I and O mark the "inverted repeats" of the mini-transposon Tn5).

Fig. 2 shows, schematically, a recombinant plasmid for expression of transaminase and of alanine dehydrogenase under the control of an arabinose inducible promoter (bla: ampicillin resistance gene; CV2025: gene for ω -transaminase from *Chromobacterium violaceum*; ald: gene for alanine dehydrogenase from *B. subtilis*; araC: transcription regulator).

Fig. 3 shows, schematically, a recombinant plasmid for the expression of LipA in *E. coli* and presentation of the enzyme on the cell surface (colE1, ColE1: replication origin; *estA**, *estA*: gene with amino acid exchange alanine for serine (codon #38), cat: chloramphenicol resistance gene; phoA: gene segment that encodes the leader sequence of alkaline phosphatase).

Fig. 4 shows determination of the activity of *C. violaceum*-transaminase from the enzyme assay. The activity was determined in duplicate (active1, active2) with a photometer. A batch without the co-substrate L-alanine (w.Cos), a batch with heat-inactivated enzyme (inactive) and a batch

from *E. coli* expression culture with empty vector (empty vector), purified similarly to the omega-TA, were used as negative controls.

Fig. 5 shows chromatograms of the substrate 12-aminomethyl laurate at the start of reference measurement (top) and after 2 h incubation with the purified transaminase (bottom).

Fig. 6 shows chromatograms of the substrate 12-aminomethyl laurate after 24 h (top) of the enzyme assay and after spiking the substrate (control, bottom).

Fig. 7 shows 6 chromatograms of the substrate 12-aminomethyl laurate after heat inactivation of the enzyme (top) and after 0h (bottom).

Fig. 8 shows the starting plasmid pGEc47, which was used as template for the amplification of alkBGTS.

Fig. 9 shows the primers used and the resultant PCR products alkBFG and alkT.

Fig.10 shows the recombinant vector pBT10, which was used for the synthesis of hydroxymethyl laurate and oxomethyl laurate.

Fig.11 shows a GC chromatogram of the 12-oxo-methyl laurate standard.

Fig.12 shows a GC chromatogram of the 12-hydroxymethyl laurate standard.

Fig.13 shows a chromatogram of the organic phase from a resting cell biotransformation in the bioreactor of methyl laurate, at time 0 min.

Fig.14 shows a chromatogram of the organic phase from a resting cell biotransformation in the bioreactor of methyl laurate, at time 150 min.

Fig.15 shows the expression vector pGJ3130 with AT3G22200.

Fig. 16 shows detection of enzyme activity by coupled enzyme assay (inactive, heat-inactivated protein; w.Cos., without addition of alanine; akt, the purified, active enzyme).

Fig. 17 shows detection of the heterologously expressed protein AT3G22200 by HPLC. Top: product after 20 min incubation at 10.8 min; bottom: reference substance at 10.8 min.

Fig.18 shows the plasmid map of the expression vector pET-21a(+) with the transaminase gene ppta5 (pPPTA5).

Fig.19 shows the plasmid map of the expression vectors pET-21a(+) with the transaminase gene psta (pPSTA).

Fig. 20 shows the amination of 5 mM 12-oxomethyl laurate with the transaminases PPTA5. For the evaluation, the peak areas of 12-oxo- and 12-aminomethyl laurate from the chromatograms obtained from neutral and acid extraction were added together and the percentage of educt or product was calculated.

Fig.21 shows the amination of 5 mM 12-oxomethyl laurate with the transaminases PSTA. For the evaluation, the peak areas of 12-oxo- and 12-aminomethyl laurate from the chromatograms obtained from neutral and acid extraction were added together and the percentage of educt or product was calculated.

EXAMPLES

A. Conversion of lauric acid or methyl laurate to laurinolactam

For the conversion of lauric acid or of methyl laurate to laurinolactam, *E. coli* is supplemented with the necessary enzymes monooxygenase, alcohol dehydrogenase, ω -transaminase, alanine dehydrogenase and a lipase. The enzymes are overexpressed in *E. coli*; the expression levels of the individual enzymes are dependent on the kinetics of the individual reactions and require optimum adjustment to one another. The expression level is adjusted by adding the appropriate amount of inductor. The expression of monooxygenase and of alcohol dehydrogenase is induced with n-octane; transaminase and alanine dehydrogenase are induced with arabinose and the lipase with IPTG.

A1. Cloning of the individual enzymes

Hydroxylation and aldehyde formation

The alkane hydroxylase system *alkBGT* from *Pseudomonas putida* GPo1 is used for the hydroxylation of lauric acid or of methyl laurate. The second step to the aldehyde is catalysed by the alcohol dehydrogenase *alkJ*.

The genes *alkBGJT* necessary for these reactions are integrated in *E. coli* by insertion into the mini-transposon Tn5 chromosomally into the genome of *E. coli* (de Lorenzo et al., *J Bacteriol.*, Vol. 172 (11), pages 6568-6572 and 6557-6567 (1990); Panke et al., *Appl and Environm. Microbiol.*, pages 5619-5623 (1999)). The genes are to be expressed under the control of the *alkB* promoter and of the positive regulator *alkS*. Transfer of the Tn5-*alkBGFJST* construct to the *E. coli* target organism is effected with the aid of the mobilizable plasmid pUT-Km (Panke et al., 1999, see above).

The *alkST* locus with the expression-relevant regulator *alkS* is organized outside of the *alkBFGHJKL* operon and arranged in the opposite direction in the genome of

Pseudomonas putida. The arrangement of the genes is preserved during cloning into the transposon-bearing plasmid. The fragments *alkST* and *alkBFGJ* are integrated into the plasmid one after another.

The genes to be cloned *alkB* (SEQ ID No. 03), *alkG* (SEQ ID No. 04) and *alkJ* (SEQ ID No. 05) are indeed organized in *P. putida* together in the operon *alkBFGHJKL*, but are separated by the gene *alkH*, which encodes an aldehyde dehydrogenase. The desired intermediate, the aldehyde of lauric acid, would be broken down again by this enzyme and the latter must therefore be excluded from the cloning of the *alkBGJ* genes.

To simplify the cloning of *alkB* and *alkG*, the gene *alkF* located between them is amplified and cloned together with *alkB* and *alkG*. *AlkF* is of no significance for the reaction that is to be catalysed. The genes *alkBFG* and *alkJ* are amplified in two separate PCR steps and fused together by SOE-PCR (Horton et al., *Gene*, Vol. 77, pages 61-68 (1989)). The OCT plasmid from *Pseudomonas putida* GPo1 serves as target DNA.

A2. Cloning strategy:

PCR amplification of the fragments *alkST* = 4077 bp (SEQ ID No. 06 (*alkS*) and SEQ ID No. 07 (*alkT*)) with *NotI* cleavage site upstream of *alkT*:

Primers

alkT-forward-*NotI* (SEQ ID No. 08)

5' ACGTAGCGGCCGCCTAATCAGGTAATTTTATAC

alkS-reverse (SEQ ID No. 09)

5' GAGCGAGCTATCTGGT

The PCR fragment is cloned into the transposon-bearing vector pUT-Km. For this, the vector is cut within *Tn5* with *NotI* and ligated with the *alkST* fragment by blunt end ligation. The recombinant plasmid is designated pUT-Km-*alkST*.

A3. Synthesis of *alkBFGJ* constructs by the SOE-PCR technique:

Synthesis of fragment 1: *alkBFG* + promoter upstream *alkB* and *NotI* cleavage site at the 5'-end (product size: 1409 bp):

Primers

alkBFG-forward-*NotI* (SEQ ID No. 10)

5' TCGTAGCGGCCGCCCAGCAGACGACGGAGCAA

alkBFG-reverse-SOE (SEQ ID No. 11)

5' ATTTTATCTTCTCGAGGCTTTTCCTCGTAGAGCACAT

Synthesis of fragment 3, *alkJ* with complementary end to *alkG* at the 5'-end and *NotI* cleavage site at the 3'-end (product size: 1723 bp):

Primers

alkJ-forward-SOE (SEQ ID No. 12)

5' TGCTCTAACGAGGAAAAGCCTCGAGAAGATAAAATGTA

alkJ-reverse-*NotI* (SEQ ID No. 13)

5' ATTGACGCGGCCGCTTACATGCAGACAGCTATCA

The two separate fragments are fused together by SOE-PCR. (3 separate PCR reactions required). The recombinant plasmid pUT-Km-*alkST* and the *alkBFGJ* construct are cut with *NotI* and ligated. The new recombinant plasmid pUT-Km-*alkBGJST* (see Fig. 1) is

transformed in *E. coli* HB101 and transferred to *E. coli* JM101 by conjugative plasmid transfer.

A4. Amination and amino donor regeneration

For amination to the ω -aminolauric acid ester and regeneration of the amino donor, the Tn5:*alkBGJST*-bearing *E. coli* strain JM101 is additionally transformed with the recombinant plasmid pBAD-CV2025-*ald*. This plasmid is based on the pBAD30 vector (Guzman et al., "Tight Regulation, Modulation, and High-Level Expression by Vectors Containing the Arabinose *P_{BAD}* Promoter", *J. Bacteriol.*, Vol. 177 (14), pages 4121-4130 (1995)). pBAD-CV2025-*ald* carries the gene for the transaminase CV2025 from *Chromobacterium violaceum* DSM30191 (SEQ ID No. 01; Kaulmann et al., *Enzyme and Microbial Technology* Vol. 41, pages 628-637 (2007) and the gene *ald*, which codes for an alanine dehydrogenase from *Bacillus subtilis subsp. Subtilis* (SEQ ID No. 02; NP_391071). The genes are under the control of an arabinose inducible promoter.

A5. Cloning strategy

PCR amplification of the transaminase gene with chromosomal DNA from *Chromobacterium violaceum* DSM30191 (product size: 1415 bp):

Primers

CV2025-forward-*SacII* (SEQ ID No. 14)

5' CGAGGAGCTCAGGAGGATCCAAGCATGCAGAAGCAACGTACG

CV2025-reverse-*KpnI* (SEQ ID No. 15)

5' GTCATGTACCCCTAAGCCAGCCCGCGCGCCT

For the cloning into the pBAD30 vector, the *forward*-primer contains a ribosome binding site in addition to the *Xba*I cleavage site. Ligation into the pBAD30 vector takes place via the cleavage sites *Sac*I and *Kpn*I. The recombinant vector is designated pBAD-CV2025.

PCR amplification of the alanine dehydrogenase gene *ald* with chromosomal DNA from *B. subtilis subsp. Subtilis* (NP_391071) (product size: 1171 bp).

Primers

*Ala*DH-forward-*Xba*I (SEQ ID No. 16)

5' ACCTATCTAGAAGGAGGACGCATATGATCATAGGGGTTTCCT

*Ala*DH-reverse-*Pst*I (SEQ ID No. 17)

5' AACCTCTGCAGTTAAGCACCCGCCAC

For the cloning into the recombinant vector pBAD-CV2025, the *forward*-primer contains a ribosome binding site in addition to the *Xba*I cleavage site. The cloning into the vector takes place via the cleavage sites *Xba*I and *Pst*I. The resultant plasmid is designated pBAD-CV2025-*ald* (see Fig. 2).

A6. Alternative cloning of the omega-transaminase gene from *Chromobacterium violaceum* DSM 30191 for codon-optimized expression in *E.coli*

The gene coding for omega-transaminase was synthesized by the company Geneart, optimized for *E. coli* codon usage (SEQ ID No. 42) and cloned into the vector pGA15 (Geneart). During synthesis of the gene, the cleavage sites *SacI* and *KpnI* were incorporated in flanking positions and, after digestion with *SacI* and *KpnI*, were cloned into the vector pGA15, linearized beforehand with *SacI* and *KpnI*. The resultant vector was digested with the restriction endonucleases *SacI* and *KpnI*, the fragment (transaminase) was purified and was ligated into the expression vector paCYCDuet-1 (Novagen). The correct plasmids were verified by restriction analysis. The resultant vector is called paCYCDuet-1::omega transaminase.

A7. Purification of heterologously expressed protein by means of 6xHis-Tag

After transformation of the vector (paCYCDuet-1::omega transaminase) in *E. coli* XL1blue, the transformed strain was cultivated in double YT-medium (dYT) with the antibiotic ampicillin (100 µg/ml) at 28°C up to a density of OD_{600 nm}=0.3-0.4. Expression takes place under the control of the P_{lac} promoter and is induced with IPTG (final concentration 1 mM). Lysis of the bacterial expression culture: 50 ml of culture was centrifuged at 2360 x g, and then resuspended in 5 ml Na-phosphate buffer (pH 8) with 5 mM EDTA, 300 mM NaCl and 1 mg/ml lysozyme, and incubated for 1h at RT. The lysate was centrifuged at 2360 x g for 10 min and the supernatant was purified on a Protino Ni-TED 2000 packed column (following the instructions of the manufacturer; Macherey-Nagel, Düren). The protein concentration was determined according to Bradford

A8. Detection of enzyme activity by coupled assay

The activity was determined in a coupled assay, in which the pyruvate formed as by-product of the transaminase reaction is reacted further in a second step, and NADH is oxidized to NAD⁺. The decrease in NADH concentration (principle: measurement of the decrease in extinction) is measured in the photometer at 340 nm and provides a measure of the activity.

Preparation	
50 mM	Na-phosphate pH 7.5
50 mM	L-alanine
100 μ M	Pyridoxal phosphate
250 μ g	12-oxomethyl dodecanoate
1.25 mM	NADH
10 U	Lactate dehydrogenase
10 μ g	heterologously expressed protein
Make up to 1 ml with doubly distilled water	

The assay was started by adding 5 μ l 12-ODME (50 mg/ml). Measurement is performed continuously every minute at 340 nm at RT up to max. 20 minutes.

Inactivated protein and a preparation without co-substrate were used for control.

Fig. 4 shows the variation in extinction, determined photometrically.

A9. Detection of the heterologously expressed protein by HPLC

Preparation	
50 mM	Na-phosphate pH 7.5
50 mM	L-alanine
100 μ M	Pyridoxal phosphate
250 μ g	12-oxomethyl dodecanoate
50 μ g	heterologously expressed protein
Make up to 500 μ l with doubly distilled water	

After incubation for 4h at RT, the reaction was stopped with 1 Vol. MeOH.

For HPLC analysis, the preparation was derivatized with o-phthalic aldehyde (oPA) and 250 μ l thereof was analysed. 50 mM NaAC pH 4:acetonitrile 4:1 (v:v) was used as solvent A.

Solvent B was acetonitrile with 5% 50 mM NaAC pH 4. The gradient was from 30% B to 60% B in 4 min, from 60% B to 100% B in 2 min. The flow rate was 1.2 ml/min.

Separation took place in an Agilent Zorbax RP18 column (Agilent Technologies, USA), the column temperature was 40°C.

Figs. 5 and 6 show the standard and the decrease of the 12-oxomethyl laurate. Heat-inactivated enzyme was used as negative control (Fig. 7).

A6. Ester cleavage

The lipase LipA (Q76D26) from *Pseudomonas fluorescens* (Kojima & Shimizu, *J. of Bioscience and Bioengin.*, Vol. 96 (3), pages 219-226 (2003)) is used for the cleavage of ω -aminomethyl laurate to ω -aminolauric acid. The gene is amplified with the primers LipA-SfiI-up and LipA-SfiI-down with chromosomal DNA from *Pseudomonas fluorescens* and cloned via the *Sfi*I cleavage sites into the vector pEST100. The recombinant plasmid is designated pEST-*lipA* (see Fig. 3).

The cloning fuses *lipA* (SEQ ID No. 18) to the signal sequence of alkaline phosphatase *phoA* and the autotransporter domain of EstA, an esterase from *P. aeruginosa*, so that the lipase is transferred across the cytoplasmic membrane and is displayed on the cell surface of *E. coli*. For the procedure see Becker et al., "A generic system for the *Escherichia coli* cell-surface display of lipolytic enzymes", *FEBS Letters* Vol. 579, pages 1177-1182 (2005). Expression takes place under the control of the P_{lac} promoter and is induced with IPTG (final concentration 1 mM) (product size: 1894 bp).

Primers

Primer *lipA*-Sfi-up (SEQ ID No. 19)

5' AACAAAAGGGCCGCAATGGCCATGGGTGTGTATGACTAC

Primer *lipA*-Sfi-down (SEQ ID No. 20)

5' TACAGGGGCCACACGGCCTCAGGCGATCACAATTCC

B Synthesis of 12-hydroxymethyl laurate and 12-oxomethyl laurate starting from methyl laurate with the AlkBGT alkane hydroxylase system from *Pseudomonas putida* GPo1

B1. Construction of the *alkBGT* Expression vectors

The construct pBT10 (Fig. 10, SEQ ID No. 31), which contains the three components alkane hydroxylase (AlkB), rubredoxin (AlkG) and rubredoxin reductase (AlkT) from *Pseudomonas putida* that are necessary for the oxidation to the aldehyde, was produced starting from the pCOM systems (Smits et al., 2001 Plasmid 64:16-24). For expression of the three genes, the *alkBFG* gene sequence was put under the control of the *alkB*-promoter and the *alkT* gene under the control of the *alkS*-promoter.

B2. Cloning strategy:

To simplify the cloning of *alkB* and *alkG*, the gene *alkF* located between them was amplified and cloned together with *alkB* and *alkG*. *AlkF* is of no significance for the reaction that is to be catalysed.

PCR amplification of the fragment *alkBFG* = 2524 bp (cf. SEQ ID No. 03 (*alkB*) and SEQ ID No. 04 (*alkG*)) with *NdeI* cleavage site upstream of *alkB* and *SalI* cleavage site downstream of *alkG*:

Primer: *alkBFG* forward (SEQ ID No. 32)

AAGGGAATTCCATATGCTTGAGAAACACAGAGTTC

Primer: *alkBFG* reverse (SEQ ID No. 33)

AAAATTCGCGTCGACAAGCGCTGAATGGGTATCGG

PCR amplification of the fragment *alkT* (2958 bp) (cf. SEQ ID No. 07 (*alkT*))

Primer *alkT* forward (SEQ ID No. 34)

TGAGACAGTGGCTGTTAGAG

Primer *alkT* reverse (SEQ ID No. 35)

TAATAACCGCTCGAGAACGCTTACCGCCAACACAG

The fragments alkBFG and alkT were amplified by PCR. The plasmid pGEc47 (Fig. 12) (Eggink et al. (1987) J. Biol. Chem. 262, 17712-17718) was used as template.

The clonings were carried out by means of the subcloning vector pSMART® HCKan (Lucigen Corporation). This additional step was necessary because direct cloning had not been successful. For this, the commercially available vector pSMART® HCKan (Lucigen), which was already linearized and provided with blunt ends, was ligated with the respective blunt-end PCR product (Fig. 9).

Next, the alkBFG fragment with the restriction enzymes NdeI and SalI and the alkT fragment with the restriction enzymes PacI and XhoI were cut out of the subcloning vectors. The fragments were separated in agarose gel (1%), cut out of the gel and isolated using a gel extraction kit.

The fragments were ligated one after another into the vector pCOM10 (Smits, T. H. M., Seeger, M. A., Witholt, B. & van Beilen, J. B. (2001) Plasmid 46, 16-24). In the first step alkBFG was inserted in pCOM10 via the cleavage sites NdeI and SalI, and in a second step alkT was then cloned via the cleavage sites PacI and XhoI.

The recombinant plasmid was first transformed in *E. coli* DH5 α . Plasmid-bearing clones were selected on kanamycin-containing LB medium. The isolated plasmid was checked by restriction analysis and sequencing. It is designated pBT10 (Fig. 10).

B3. Biotransformation in the bioreactor of methyl laurate to hydroxymethyl laurate and 12-oxomethyl laurate

For the biotransformation, the plasmid pBT10 was transformed by heat shock at 42°C for 2 min in the chemically competent strain *E. coli* W3110. For the synthesis of hydroxymethyl laurate and 12-oxomethyl laurate, *E. coli* W3110-pBT10 was cultivated overnight at 30°C and 180 rpm in M9 medium and harvested. The biomass was taken up in M9 medium with 0.5% glucose up to OD₄₅₀ = 0.2. After a growth time of 4 h, expression was induced with dicyclopropyl ketone and it was incubated for a further 4 hours. The cells were centrifuged,

the cell pellet was resuspended in KPi-buffer (50 mM, pH 7.4) and put in a bioreactor. A biomass concentration of about 1.8 gCDW/L was established. Stirring vigorously (1500 min⁻¹), the substrate methyl laurate in the ratio 1:3 was added to the cell suspension (100 ml cell suspension, 50 ml methyl laurate). The temperature was kept constant at 30°C. Formation of the products hydroxymethyl laurate and 12-oxomethyl laurate was detected by GC analysis of the reaction mixture. For this, a sample was taken after 0 min as negative control (Fig. 13) and after 150 min (Fig. 14) from the organic phase of the reaction mixture, and was analysed by GC (Thermo Trace GC Ultra). The column was a Varian Inc. FactorFour™ VF-5m, length: 30 m, film thickness: 0.25 µm, inside diameter: 0.25 mm.

Analysis conditions:

Furnace temperature	80 – 280°C
Ramp	15°C/min
Split ratio	15
Injection volume	1 µl
Carrier flow	1.5 ml/min
PTV injector	80 – 280°C at 15°C/s
Detector base temperature:	320°C

The detection of 12-oxomethyl laurate (Fig. 11) and 12-hydroxymethyl laurate (Fig. 12) was demonstrated by injection of the pure substances.

C Conversion of 12-oxomethyl laurate to 12-aminomethyl laurate

C 1: Isolation and expression of an aminotransferase from *Arabidopsis thaliana*

A known aminotransferase from *Arabidopsis thaliana* was analysed. Surprisingly, 4-aminobutyrate transaminase (at3g22200, SEQ ID No. 38) displayed an activity of about 14 U/mg heterologously expressed protein versus 12-oxomethyl dodecanoate. The product 12-aminomethyl dodecanoate was confirmed by HPLC.

Unless stated otherwise, all methods were carried out in accordance with the protocols in Sambrook, J., Fritsch, E. F., & Maniatis, T (1989). Molecular Cloning, 2nd Ed. New York: Cold Spring Harbor Laboratory Press.

Isolation of 4-aminobutyrate transaminase from *A.thaliana* (at3g22200)

The RNA was isolated with the RNeasy Mini Kit from a whole, flowering plant of the species *A. thaliana* following the instructions of the manufacturer: QIAGEN GmbH, Hilden.

Then cDNA synthesis was carried out with the RT Skript Kit (USB Europe GmbH, Staufien) following the manufacturer's instructions. RNA quality/quantity determination was performed by Nanodrop following the instructions of the manufacturer (Thermo Fisher Scientific Inc. Waltham USA).

PCR from *A.thaliana* cDNA for insertion of cleavage sites

Using the following primers, the DNA coding for the 4-aminobutyrate transaminase with SEQ ID No. 39 was cloned in the NaeI, BamHI digested vector

Forward-primer inserts protease cleavage site and NaeI, SEQ ID No. 36

GCCGGCGAGAACCTGTACTTTCAGATGGCAAGTAAGTATGCCACTTG

Reverse-primer inserts BamHI, SEQ ID No. 37

GGATCCTCACTTCTTGTGCTGAGCCTTG

PCR was carried out according to the following protocol:

Preparation	Programme
2 µl cDNA	95°C 3 min
5 µl 10x Pfu buffer MgSO ₄	94°C 45 sec
5 µl dNTPs 2 mM	58°C 1 min 30 cycles } 72°C 4 min
2 µl primer forward 10 µM	72°C 10 min
2 µl primer reverse 10 µM	
0.5 µl Pfu	
36.5 µl H ₂ O	

The resultant PCR product was purified with the NucleoSpin® Extract II Kit (Macherey-Nagel, Germany, following the manufacturer's instructions).

Expression of the heterologous protein

Using the PCR product described above, the vector pGJ3130 (Fig. 15, SEQ ID No. 43) was produced by standard methods of molecular biology and transformed in *E. coli* XL1blue. The transformed *E. coli* strain was cultivated in double YT-medium (dYT) with the antibiotic ampicillin (100 µg/ml) and addition of 0.5 mM IPTG at 28°C up to a density of OD600 nm=0.3-0.4.

Purification of heterologously expressed protein by means of 6xHis-Tag

Lysis of the bacterial expression culture: 50 ml culture was centrifuged at 2360 x g, and then resuspended in 5 ml Na-phosphate buffer (pH 8) with 5 mM EDTA, 300 mM NaCl and 1 mg/ml lysozyme, and incubated for 1h at RT. The lysate was centrifuged at 2360 x g for 10 min and the supernatant was purified on a Protino Ni-TED 2000 packed column (following the instructions of the manufacturer; Macherey-Nagel, Düren). The protein concentration was determined according to Bradford.

Detection of enzyme activity by means of a coupled assay

The activity was determined in a coupled assay, in which the pyruvate that formed as by-product of the transaminase reaction is reacted further in a second step, and NADH is oxidized to NAD⁺. The decrease in NADH concentration (principle: measurement of the decrease in extinction) is measured in the photometer at 340 nm and provides a measure of the activity.

Preparation	
50 mM	Na-phosphate pH 7.5
50 mM	L-alanine
100 µM	Pyridoxal phosphate
250 µg	12-oxomethyl dodecanoate
1.25 mM	NADH

10 U	Lactate dehydrogenase
10 μ g	heterologously expressed protein
Make up to 1 ml with doubly distilled water	

The assay was started by adding 5 μ l 12-ODME (50 mg/ml). Measurement is performed continuously every minute at 340 nm at RT for up to max. 20 minutes.

Inactivated protein and a preparation without co-substrate were used as the control.

Fig. 16 shows the variation in extinction, determined photometrically.

Detection of the heterologously expressed protein by HPLC

Preparation	
50 mM	Na-phosphate pH 7.5
50 mM	L-alanine
100 μ M	Pyridoxal phosphate
250 μ g	12-oxomethyl dodecanoate
50 μ g	heterologously expressed protein
Make up to 500 μ l with doubly distilled water	

After incubation for 4h at RT, the reaction was stopped with 1 Vol. MeOH

For the HPLC analysis, the preparation was derivatized with o-phthalic aldehyde (oPA) and 250 μ l was analysed. 50 mM NaAC pH 4:acetonitrile 4:1 (v:v) was used as solvent A. Solvent B was acetonitrile with 5% 50 mM NaAC pH 4. The gradient was from 30% B to 60% B in 4 min, from 60% B to 100% B in 2 min. The flow rate was 1.2 ml/min.

Separation took place in an Agilent Zorbax RP18 column (Agilent Technologies, USA), the column temperature was 40°C. Fig. 17 (top) shows the formation of 12-aminomethyl laurate. The reference sample is shown at the bottom in Fig. 17.

D Amination of 12-oxomethyl laurate with PPTA5 and PSTA from *Pseudomonas*

D1. Cloning of PPTA5 and PSTA

The strains *E. coli* BL21(DE3)/PPTA5 and *E. coli* BL21(DE3)/PSTA were used for the amination of 12-oxomethyl laurate. These strains were constructed as follows. The expression vector pET-21a(+) (Novagen) was selected for the cloning of both transaminase genes. For the PPTA5 gene, SEQ ID No. 40, primers were constructed, which were intended to add the restriction cleavage sites NdeI and XhoI to the ends of the gene; primer PPTA5_NdeI: GGAATTCCATATGAGCGTCAACAACCCGCAAACCCG (SEQ ID No. 44) and

Primer PPTA5_XhoI: CCGCTCGAGTTATCGAATCGCCTCAAGGGTCAGGTCC (SEQ ID No. 45). For the psta gene, SEQ ID No. 41, primers with NdeI and BamHI at the ends; primer PSTA_NdeI:

GGAATTCCATATGAGCGATTTCGAAACCCTGCACTGGC (SEQ ID No. 46) and

Primer PSTA_BamHI: CGCGGATCCTCAGCCCAGCACATCCTTGGCTGTCG (SEQ ID No. 47)

These primers were used in PCRs. The purified PCR products and the vector pET-21a(+) were then submitted to restriction with the restriction enzymes NdeI and XhoI or NdeI and BamHI. The cut vector was dephosphorylated with alkaline phosphatase from shrimp. The vector cut with NdeI and XhoI and the PPTA5 gene, and the vector cut with NdeI and BamHI and the psta gene, were, after ligation with T4 DNA ligase, transformed with the competent expression strain *E. coli* XL1-Blue. After some clones had been grown, the plasmids were isolated and then underwent restriction and gel electrophoretic analysis. The transaminase sequences of the clones obtained (pPPTA5 or pPSTA) were confirmed by sequence analysis. Fig. 18 shows the plasmid maps of the expression vectors.

D2. Expression of PPTA5 and PSTA

For expression, the vectors pPPTA5 and pPSTA were transformed in competent *E. coli* BL21(DE3) cells. One individual colony of each was inoculated in 5 ml LB-Amp medium (ampicillin concentration 100 µg/ml) and shaken overnight at 37°C. Then 1% was inoculated in 200 ml LB-Amp medium, shaken at 37°C and after reaching an OD₆₀₀ of 0.5, gene expression was induced with 0.5 mM IPTG. After shaking for 20 hours at 30°C, the cells were harvested and stored at -20°C.

For digestion of the strains *E. coli* BL21(DE3)/pPPTA5 and *E. coli* BL21(DE3)/pPSTA, 0.4 g of cells from each were processed with 100 mM Tris-HCl buffer pH 7.2 to 25% cell suspensions, which were treated twice for 90 sec with ultrasound (Bandelin Sonoplus HD2070; probe MS73; 50% intensity). After centrifugation, the supernatants were removed. The raw extracts obtained were used in conversions of 12-oxomethyl laurate. The 400 μ l preparations contained 5 mM 12-oxomethyl laurate, dissolved in N,N-dimethylformamide, 500 mM DL-alanine, 1 mM pyridoxal-5'-phosphate and 80 μ l raw extract in 10 mM Kpi-buffer pH 7.0. It was shaken at 25°C. After specified times, 20 μ l samples were taken from each, one portion was made alkaline with 1 μ l 1% NaOH solution and was shaken out with 100 μ l ethyl acetate. The organic phases were investigated by gas chromatography (gas chromatograph from Perkin Elmer, Clarus 500 with flame ionization detector). For this, an Optima 5-column (0.25 μ m, 30 m, 0.25 mm, Macherey-Nagel) was used with programme:

80°C	
25°C/min	180°C
5°C/min	215°C
20°C/min	280°C

The retention times of 12-oxo- and 12-aminomethyl laurate are 7.2 and 7.7 min, respectively.

The results of the reactions are presented in Figs. 20 and 21. For the evaluation, the peak areas of 12-oxo- and 12-aminomethyl laurate from the chromatograms obtained for neutral and acid extraction were added together and the percentage of educt or product was calculated.

PATENT CLAIMS

1. Cell, which has been genetically modified relative to its wild type so that, in comparison with its wild type, it is able to produce more ω -aminocarboxylic acids, more ω -aminocarboxylic acid esters or more lactams derived from ω -aminocarboxylic acids, starting from carboxylic acids or carboxylic acid esters.
2. Cell according to Claim 1, characterized in that the cell has increased activity, in comparison with its wild type, of at least one of the following enzymes:
 - i) an enzyme E_I, which catalyses the conversion of carboxylic acids or carboxylic acid esters to the corresponding ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters;
 - ii) an enzyme E_{II}, which catalyses the conversion of ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters to the corresponding ω -oxocarboxylic acids or ω -oxocarboxylic acid esters;
 - iii) an enzyme E_{III}, which catalyses the conversion of ω -oxocarboxylic acids or ω -oxocarboxylic acid esters to the corresponding ω -aminocarboxylic acids or ω -aminocarboxylic acid esters.
3. Cell according to Claim 2, characterized in that the cell has increased activity of all the enzymes E_I, E_{II} and E_{III}.
4. Cell according to Claim 2 or 3, characterized in that
 - the enzyme E_I is an alkane monooxygenase, or
 - the enzyme E_{II} is an alkane monooxygenase, an alcohol dehydrogenase, or an alcohol oxidase, or
 - the enzyme E_{III} is an ω -transaminase.
5. Cell according to one of Claims 2 to 4, characterized in that the enzyme E_I is the *alkBGT*-encoded alkane monooxygenase from *Pseudomonas putida* GPo1.

6. Cell according to one of Claims 2 to 5, characterized in that the enzyme E_{II} is encoded by the *alkJ* gene from *Pseudomonas putida* GPo1.
7. Cell according to one of Claims 2 to 6, characterized in that the enzyme E_{III} is the ω -transaminase CV2025 from *Chromobacterium violaceum* DSM30191.
8. Cell according to one of the preceding claims, characterized in that the expression of an enzyme E_{IV}, which catalyses the conversion of ω -aminocarboxylic acid esters to the corresponding ω -aminocarboxylic acids, is increased in the cell.
9. Cell according to Claim 8, characterized in that the enzyme E_{IV} is the lipase LipA (Q76D26) from *Pseudomonas fluorescens*, which is secreted by the cell.
10. The cell according to one of the preceding claims, characterized in that the expression of an enzyme E_V, which catalyses the conversion of ω -aminocarboxylic acids to the corresponding lactams, is increased in the cell.
11. Cell according to Claim 10, characterized in that the enzyme E_V is secreted by the cell.
12. Cell according to one of the preceding claims, characterized in that the cell is a genetically modified *Escherichia coli* cell, a genetically modified *Corynebacterium glutamicum* cell or a genetically modified *Pseudomonas putida* cell.
13. A method for the production of a genetically modified cell, comprising the process step of increasing the activity of at least one of the following enzymes:
 - i) an enzyme E_I, which catalyses the conversion of carboxylic acids or carboxylic acid esters to the corresponding ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters;
 - ii) an enzyme E_{II}, which catalyses the conversion of ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters to the corresponding ω -oxocarboxylic acids or ω -oxocarboxylic acid esters;

- iv) an enzyme E_{III}, which catalyses the conversion of ω -oxocarboxylic acids or ω -oxocarboxylic acid esters to the corresponding ω -aminocarboxylic acids or ω -aminocarboxylic acid esters in a cell.
14. Method according to Claim 13, characterized in that in addition to the increase in activity of the enzymes E_I, E_{II} or E_{III}, also the activity of an enzyme E_{IV}, which catalyses the conversion of ω -aminocarboxylic acid esters to the corresponding ω -aminocarboxylic acids, and/or an enzyme E_V, which catalyses the conversion of ω -aminocarboxylic acids to the corresponding lactams, is increased by increasing the expression of these enzymes and in that the enzymes E_{IV} and/or E_V are secreted by the cell.
15. Genetically modified cell, obtainable by the method according to Claim 13 or 14.
16. Method for the production of ω -aminocarboxylic acids, of ω -aminocarboxylic acid esters or of lactams derived from ω -aminocarboxylic acids, containing the process steps:
- I) contacting a cell according to one of Claims 1 to 12 or 15 with a culture medium containing a carboxylic acid or a carboxylic acid ester or with a culture medium contiguous with an organic phase containing a carboxylic acid or a carboxylic acid ester in conditions that make it possible for the cell to form ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids, starting from carboxylic acid or from carboxylic acid esters;
- II) optionally isolation of the resultant ω -aminocarboxylic acids, the resultant ω -aminocarboxylic acid esters or the lactams derived from ω -aminocarboxylic acids.
17. Method according to Claim 16, characterized in that the ω -aminocarboxylic acid esters formed in step I) are converted in another process step by conventional chemical methods to ω -aminocarboxylic acids.

18. Method according to Claim 16 or 17, characterized in that the cell is a genetically modified *Escherichia coli* cell, a genetically modified *Corynebacterium glutamicum* cell or a genetically modified *Pseudomonas putida* cell.
19. Method according to one of Claims 16 to 18, characterized in that the culture medium used in step I) contains amino acids, which function as amine donor in the transaminase-catalysed conversion of the ω -oxocarboxylic acids or the ω -oxocarboxylic acid esters to the corresponding ω -aminocarboxylic acids or ω -aminocarboxylic acid esters.
20. Method according to one of Claims 16 to 19, characterized in that the method is carried out in a two-phase system, containing
 - A) an aqueous phase, and
 - B) an organic phase,where the formation of the ω -aminocarboxylic acids, the ω -aminocarboxylic acid esters or the lactams derived from ω -aminocarboxylic acids by the cells in step I) takes place in the aqueous phase and the resultant ω -aminocarboxylic acids, the resultant ω -aminocarboxylic acid esters or the resultant lactams derived from ω -aminocarboxylic acids accumulate in the organic phase.
21. Method according to one of Claims 16 to 20, characterized in that the isolation of the resultant ω -aminocarboxylic acids, the resultant ω -aminocarboxylic acid esters or the lactams derived from ω -aminocarboxylic acids takes place by an at least two-stage purification process, comprising
 - a) an extraction step, in which the ω -aminocarboxylic acids, the ω -aminocarboxylic acid esters or the lactams derived from ω -aminocarboxylic acids are extracted from the culture medium, and
 - b) a fine purification step, in which the extract obtained in step a) is purified further by distillation methods or additional extraction processes, obtaining an ω -

aminocarboxylic acid phase, an ω -aminocarboxylic acid ester phase or a lactam phase with a purity of at least 99.8%.

22. Method according to Claim 21, characterized in that the extraction in step a) is a reactive extraction.
23. Method according to one of Claims 16 to 22, characterized in that the carboxylic acid is lauric acid or the carboxylic acid ester is methyl laurate and in that the lauric acid or the methyl laurate is converted in step II) to laurinlactam.
24. ω -Aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids, obtainable by a method according to one of Claims 16 to 23.
25. Laurinlactam, obtainable by a method according to Claim 23.
26. Method for the production of polyamides based on ω -aminocarboxylic acids, comprising the process steps:
 - (α 1) production of ω -aminocarboxylic acids by a method according to one of Claims 16 to 23;
 - (α 2) polymerization of the ω -aminocarboxylic acid, obtaining a polyamide.
26. Method for the production of polyamides based on lactams, comprising the process steps:
 - (β 1) production of lactams by a method according to one of Claims 16 to 23;
 - (β 2) ring opening polymerization or polycondensation of the laurinlactam, obtaining a polyamide.
26. Polyamide, obtainable by a method according to Claim 25 or 26.

SEQUENCE LISTING

<110> Evonik Degussa GmbH

<120> Recombinant cells which produce omega-aminocarboxylic acids, omega-aminocarboxylic esters or lactams thereof

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gtagattggg ctggtggtga ccttaataag atccaaattg atgattcgat gcgagaaacc	1140
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tcgtag	1206

<210> 4
 <211> 519
 <212> DNA
 <213> *Pseudomonas putida*

<400> 4	
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gattgcgccg ttcgagacaa gcttgacttc atgttaattg agagcggcgt aggtgaaaag	180
ggcgtcacct caaccatac ttgcgcaaat ttatccgagg ttagtggcac aagtttaact	240
gctgaagcag tggttgcgcc gacaagctta gagaaattgc ctagtgccga cgttaaaggc	300
caagatctat ataaaactca acctccaagg tctgatgccc aaggcgggaa agcatacttg	360
aagtggatat gtattacttg tggccatata tatgatgagg cgttgggcga tgaggccgag	420
ggttttactc caggtactcg ctttgaggat attcctgatg actgggtgctg tccggattgc	480
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<210> 5
 <211> 1677
 <212> DNA
 <213> Pseudomonas putida

<400> 5
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 ctaattcata tgccgttagg tattgctttg ctttcaaata gtaaaaagtt gaattgggct 180
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 taccacgcat gggagcaggc ggccggccgc tactgggggtt ggtaccgggc tcttgagttg 360
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 gaaaatgcgg cagaaataat tatgcggaat cttgatgtgg aagcattaga ggctagcgct 1620

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<210> 6
 <211> 2649
 <212> DNA
 <213> Pseudomonas putida

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 cttgcgttcg agtggctacg ccacagaaca gccggacgtc ctgcagtgtg gctttcttta 240
 agagccagtt cttacagtga atttgatatc tgcgcagaga ttattgagca gcttgaaact 300
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 gaccttgcat ctagtctttg gcagagcacc tcgaataacg agatagaaac gctagtttgt 420
 ttggataata ttaatcatga cttagacttg ccgttggtgc acgcacttat ggagtttatg 480
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gcttttagtc ttccgcgaat agttgagatt ggaaagtccg cagagaataa agctgacgct	2460
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caaatagcaa caaatatgca tgtcacgaa gatgctataa agtggcatat gaggaaaata	2580
tttgccacct tgaatgtagt gaatcgacg caagcaacaa ttgaagctga gcgtcaagga	2640
attatctaa	2649

<210> 7
 <211> 1158
 <212> DNA
 <213> *Pseudomonas putida*

<400> 7	
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cggcctctc tatccaaggc ttttctgaca agtgagattg cagaatccgc agtgccatta	180
aagccagaag gtttttatat gaataacaat attaccattt cgttaaatac accgattgta	240
tcaatcgacg tggggcgtaa gatagtttct tctaaagatg gaaaagaata cgcgtatgaa	300
aaattgattc ttgcaacacc tgctagcgca cgtagggttaa cctgcgaggg gtctgaactg	360
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gagagtgcgt ctgttgttgt gttgggcggc ggagtaatcg ggcttgaagt cgcctcagct	480

gcggtgggct tagggaagag ggtcacagtg atagaagcca ccccgctgt aatggcgcg	540
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ccagagctag agctggcaac tgaggcggcc cttgaagtga gtaatgggtgt tgtggctgat	780
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aatccttttt ggggaacgat ggtacgttta gagacaattc ataatgcggt tacacacgct	900
caaattgtcg caagtagcat ctgtggcaca tcaacaccag caccaacccc accacggttc	960
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ctcgttggtg caattaataa cgaaactctt gaactagaag tccttgcgta caagcaggag	1080
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ataaaattac ctgattag	1158

<210> 8
 <211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer

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acgtagcggc cgcctaataca ggtaatttta tac	33

<210> 9
 <211> 16
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer

<400> 9	
gagcgagcta tctggt	16

<210> 10
 <211> 32
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer

<400> 10	
tcgtagcggc cgcccagcag acgacggagc aa	32

<210> 11
 <211> 37
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Primer

 <400> 11
 attttatctt ctcgaggctt ttctctgtag agcacat 37

 <210> 12
 <211> 38
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Primer

 <400> 12
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 <210> 13
 <211> 34
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Primer

 <400> 13
 attgacgcgg ccgcttacat gcagacagct atca 34

 <210> 14
 <211> 42
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Primer

 <400> 14
 cgaggagctc aggaggatcc aagcatgcag aagcaacgta cg 42

 <210> 15
 <211> 31
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Primer

 <400> 15
 gtcatgtacc cctaagccag cccgcgcgcc t 31

<210> 16
 <211> 41
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer

<400> 16
 acctatctag aaggaggacg catatgatca taggggttcc t 41

<210> 17
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer

<400> 17
 aacctctgca gttaagcacc cgccac 26

<210> 18
 <211> 1853
 <212> DNA
 <213> Pseudomonas fluoreszenz

<400> 18
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 gccatggcga tcacgctgta ttcctaccac aacctcgata acggttttgc cgccggttat 120
 agcacaacgg ttttggcctt ggcttgccgg cgacgctggt caccggcgtt ctcggcggta 180
 ccgattccca gggcgtcate ccgggcatte cgtggaatcc cgattcggaa aaactcgccc 240
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 tggttggcgt ttcgctgggg agtttgagtg gcgatggaat tgtgatcgcc tga 1853

<210> 19
 <211> 503
 <212> PRT
 <213> *Pseudomonas putida* W619

<400> 19

Met Ser Lys Ser Leu Thr Trp Ala Arg Gly Ser Gly Arg Arg Leu Ile
 1 5 10 15

Ser Leu Val Leu Arg Tyr Leu Lys Ile Arg Asn Val Val Phe Phe Arg
 20 25 30

Leu Tyr Pro Arg Ala Asn Leu Ala Ala Thr Thr Ser Ile Val Ala Tyr
 35 40 45

Ser Phe Phe Glu Glu Pro Pro Met Asn Met Pro Glu Thr Ala Pro Ala
 50 55 60

Gly Ile Ala Ser Gln Leu Lys Leu Asp Ala His Trp Met Pro Tyr Thr
 65 70 75 80

Ala Asn Arg Asn Phe His Arg Asp Pro Arg Leu Ile Val Ala Ala Glu
 85 90 95

Gly Asn Tyr Leu Val Asp Asp Lys Gly Arg Arg Ile Phe Asp Ala Leu
 100 105 110

Ser Gly Leu Trp Thr Cys Gly Ala Gly His Thr Arg Lys Glu Ile Thr
 115 120 125

Glu Ala Val Ala Arg Gln Leu Gly Thr Leu Asp Tyr Ser Pro Ala Phe
 130 135 140

Gln Phe Gly His Pro Leu Ser Phe Gln Leu Ala Glu Lys Ile Thr Ala
 145 150 155 160

Leu Thr Pro Gly Asp Leu Asn His Val Phe Tyr Thr Asn Ser Gly Ser
 165 170 175

Glu Cys Ala Asp Thr Ala Leu Lys Met Val Arg Ala Tyr Trp Arg Leu
 180 185 190

Lys Gly Gln Ala Thr Lys Thr Lys Ile Ile Gly Arg Ala Arg Gly Tyr
 195 200 205

His Gly Val Asn Ile Ala Gly Thr Ser Leu Gly Gly Val Asn Gly Asn
 210 215 220

Arg Lys Met Phe Gly Gln Leu Leu Asp Val Asp His Leu Pro His Thr
 225 230 235 240

Val Leu Pro Val Asn Ala Phe Ser Lys Gly Leu Pro Glu Glu Gly Gly
 245 250 255

Ile Ala Leu Ala Asp Glu Met Leu Lys Leu Ile Glu Leu His Asp Ala
 260 265 270

Ser Asn Ile Ala Ala Val Ile Val Glu Pro Leu Ala Gly Ser Ala Gly
 275 280 285

Val Leu Pro Pro Pro Lys Gly Tyr Leu Lys Arg Leu Arg Glu Ile Cys
 290 295 300

Thr Gln His Asn Ile Leu Leu Ile Phe Asp Glu Val Ile Thr Gly Phe
 305 310 315 320

Gly Arg Met Gly Ala Met Thr Gly Ala Glu Ala Phe Gly Val Thr Pro
 325 330 335

Asp Leu Met Cys Ile Ala Lys Gln Val Thr Asn Gly Ala Ile Pro Met
 340 345 350

Gly Ala Val Ile Ala Ser Ser Glu Ile Tyr Gln Thr Phe Met Asn Gln
 355 360 365

Pro Thr Pro Glu Tyr Ala Val Glu Phe Pro His Gly Tyr Thr Tyr Ser
 370 375 380

Ala His Pro Val Ala Cys Ala Ala Gly Ile Ala Ala Leu Asp Leu Leu
 385 390 395 400

Gln Arg Glu Asn Leu Val Gln Ser Ala Ala Glu Leu Ala Pro His Phe
 405 410 415

Glu Lys Leu Leu His Gly Val Lys Gly Thr Lys Asn Val Val Asp Ile
 420 425 430

Arg Asn Tyr Gly Leu Ala Gly Ala Ile Gln Ile Ala Ala Arg Asp Gly
 435 440 445

Asp Ala Ile Val Arg Pro Tyr Glu Val Ala Met Lys Leu Trp Lys Ala
 450 455 460

Gly Phe Tyr Val Arg Phe Gly Gly Asp Thr Leu Gln Phe Gly Pro Thr
 465 470 475 480

Phe Asn Thr Thr Pro Gln Gln Leu Asp Arg Leu Phe Asp Ala Val Gly
 485 490 495

Glu Asn Leu Asn Leu Ile Asp
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<210> 20

<211> 460

<212> PRT

<213> Pseudomonas aeruginosa PA01

<400> 20

Met Thr Ala Gln Leu Asn Pro Gln Arg Asp Thr Arg Asp Tyr Gln Gln
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Leu Asp Ala Ala His His Ile His Ala Phe Leu Asp Gln Lys Ala Leu
 20 25 30

Asn Arg Glu Gly Pro Arg Val Met Val Arg Gly Asp Gly Leu Gln Leu

35

40

45

Trp Asp Asn Asp Gly Lys Arg Tyr Leu Asp Gly Met Ser Gly Leu Trp
50 55 60

Cys Thr Asn Leu Gly Tyr Gly Arg Gln Asp Leu Ala Ala Ala Ala Ser
65 70 75 80

Arg Gln Leu Glu Gln Leu Pro Tyr Tyr Asn Met Phe Phe His Thr Thr
85 90 95

His Pro Ala Val Val Glu Leu Ser Glu Met Leu Phe Ser Leu Leu Pro
100 105 110

Asp His Tyr Ser His Ala Ile Tyr Thr Asn Ser Gly Ser Glu Ala Asn
115 120 125

Glu Val Leu Ile Arg Thr Val Arg Arg Tyr Trp Gln Ile Leu Gly Lys
130 135 140

Pro Gln Lys Lys Ile Met Ile Gly Arg Trp Asn Gly Tyr His Gly Ser
145 150 155 160

Thr Leu Gly Ser Thr Ala Leu Gly Gly Met Lys Phe Met His Glu Met
165 170 175

Gly Gly Met Leu Pro Asp Phe Ala His Ile Asp Glu Pro Tyr Trp Tyr
180 185 190

Ala Asn Gly Gly Glu Leu Ser Pro Ala Glu Phe Gly Arg Arg Ala Ala
195 200 205

Leu Gln Leu Glu Glu Lys Ile Leu Glu Leu Gly Ala Glu Asn Val Ala
210 215 220

Ala Phe Val Ala Glu Pro Phe Gln Gly Ala Gly Gly Met Ile Phe Pro
225 230 235 240

Pro Gln Ser Tyr Trp Pro Glu Ile Gln Arg Ile Cys Arg Gln Tyr Asp
245 250 255

Val Leu Leu Cys Ala Asp Glu Val Ile Gly Gly Phe Gly Arg Thr Gly
260 265 270

Glu Trp Phe Ala His Glu His Phe Gly Phe Gln Pro Asp Thr Leu Ser
275 280 285

Ile Ala Lys Gly Leu Thr Ser Gly Tyr Ile Pro Met Gly Gly Leu Val
 290 295 300
 Leu Gly Lys Arg Ile Ala Glu Val Leu Val Glu Gln Gly Gly Val Phe
 305 310 315 320
 Ala His Gly Leu Thr Tyr Ser Gly His Pro Val Ala Ala Ala Val Ala
 325 330 335
 Ile Ala Asn Leu Lys Ala Leu Arg Asp Glu Gly Val Val Thr Arg Val
 340 345 350
 Arg Glu Glu Thr Gly Pro Tyr Leu Gln Arg Cys Leu Arg Glu Val Phe
 355 360 365
 Gly Asp His Pro Leu Val Gly Glu Val Gln Gly Ala Gly Phe Val Ala
 370 375 380
 Ala Leu Gln Phe Ala Glu Asp Lys Val Thr Arg Lys Arg Phe Ala Asn
 385 390 395 400
 Glu Asn Asp Leu Ala Trp Arg Cys Arg Thr Ile Gly Phe Glu Glu Gly
 405 410 415
 Val Ile Ile Arg Ser Thr Leu Gly Arg Met Ile Met Ala Pro Ala Leu
 420 425 430
 Val Ala Gly Arg Ala Glu Ile Asp Glu Leu Ile Asp Lys Thr Arg Ile
 435 440 445
 Ala Val Asp Arg Thr Ala Arg Glu Ile Gly Val Leu
 450 455 460

<210> 21
 <211> 448
 <212> PRT
 <213> Pseudomonas aeruginosa PA01

<400> 21

Met Asn Gln Pro Leu Asn Val Ala Pro Pro Val Ser Ser Glu Leu Asn
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Leu Arg Ala His Trp Met Pro Phe Ser Ala Asn Arg Asn Phe Gln Lys
 20 25 30

Asp Pro Arg Ile Ile Val Ala Ala Glu Gly Ser Trp Leu Thr Asp Asp
 35 40 45

Lys Gly Arg Lys Val Tyr Asp Ser Leu Ser Gly Leu Trp Thr Cys Gly
 50 55 60

Ala Gly His Ser Arg Lys Glu Ile Gln Glu Ala Val Ala Arg Gln Leu
 65 70 75 80

Gly Thr Leu Asp Tyr Ser Pro Gly Phe Gln Tyr Gly His Pro Leu Ser
 85 90 95

Phe Gln Leu Ala Glu Lys Ile Ala Gly Leu Leu Pro Gly Glu Leu Asn
 100 105 110

His Val Phe Phe Thr Gly Ser Gly Ser Glu Cys Ala Asp Thr Ser Ile
 115 120 125

Lys Met Ala Arg Ala Tyr Trp Arg Leu Lys Gly Gln Pro Gln Lys Thr
 130 135 140

Lys Leu Ile Gly Arg Ala Arg Gly Tyr His Gly Val Asn Val Ala Gly
 145 150 155 160

Thr Ser Leu Gly Gly Ile Gly Gly Asn Arg Lys Met Phe Gly Gln Leu
 165 170 175

Met Asp Val Asp His Leu Pro His Thr Leu Gln Pro Gly Met Ala Phe
 180 185 190

Thr Arg Gly Met Ala Gln Thr Gly Gly Val Glu Leu Ala Asn Glu Leu
 195 200 205

Leu Lys Leu Ile Glu Leu His Asp Ala Ser Asn Ile Ala Ala Val Ile
 210 215 220

Val Glu Pro Met Ser Gly Ser Ala Gly Val Leu Val Pro Pro Val Gly
 225 230 235 240

Tyr Leu Gln Arg Leu Arg Glu Ile Cys Asp Gln His Asn Ile Leu Leu
 245 250 255

Ile Phe Asp Glu Val Ile Thr Ala Phe Gly Arg Leu Gly Thr Tyr Ser
 260 265 270

Gly Ala Glu Tyr Phe Gly Val Thr Pro Asp Leu Met Asn Val Ala Lys

275

280

285

Gln Val Thr Asn Gly Ala Val Pro Met Gly Ala Val Ile Ala Ser Ser
 290 295 300

Glu Ile Tyr Asp Thr Phe Met Asn Gln Ala Leu Pro Glu His Ala Val
 305 310 315 320

Glu Phe Ser His Gly Tyr Thr Tyr Ser Ala His Pro Val Ala Cys Ala
 325 330 335

Ala Gly Leu Ala Ala Leu Asp Ile Leu Ala Arg Asp Asn Leu Val Gln
 340 345 350

Gln Ser Ala Glu Leu Ala Pro His Phe Glu Lys Gly Leu His Gly Leu
 355 360 365

Gln Gly Ala Lys Asn Val Ile Asp Ile Arg Asn Cys Gly Leu Ala Gly
 370 375 380

Ala Ile Gln Ile Ala Pro Arg Asp Gly Asp Pro Thr Val Arg Pro Phe
 385 390 395 400

Glu Ala Gly Met Lys Leu Trp Gln Gln Gly Phe Tyr Val Arg Phe Gly
 405 410 415

Gly Asp Thr Leu Gln Phe Gly Pro Thr Phe Asn Ala Arg Pro Glu Glu
 420 425 430

Leu Asp Arg Leu Phe Asp Ala Val Gly Glu Ala Leu Asn Gly Ile Ala
 435 440 445

<210> 22

<211> 444

<212> PRT

<213> Pseudomonas aeruginosa PA01

<400> 22

Met Thr Met Asn Asp Glu Pro Gln Ser Ser Ser Leu Asp Asn Phe Trp
 1 5 10 15

Met Pro Phe Thr Ala Asn Arg Gln Phe Lys Ala Arg Pro Arg Leu Leu
 20 25 30

Glu Ser Ala Glu Gly Ile His Tyr Ile Ala Gln Gly Gly Arg Arg Ile
 35 40 45

Leu Asp Gly Thr Ala Gly Leu Trp Cys Cys Asn Ala Gly His Gly Arg
 50 55 60

Arg Glu Ile Ser Glu Ala Val Ala Arg Gln Ile Ala Thr Leu Asp Tyr
 65 70 75 80

Ala Pro Pro Phe Gln Met Gly His Pro Leu Pro Phe Glu Leu Ala Ala
 85 90 95

Arg Leu Thr Glu Ile Ala Pro Pro Ser Leu Asn Lys Val Phe Phe Thr
 100 105 110

Asn Ser Gly Ser Glu Ser Ala Asp Thr Ala Leu Lys Ile Ala Leu Ala
 115 120 125

Tyr Gln Arg Ala Ile Gly Gln Gly Thr Arg Thr Arg Leu Ile Gly Arg
 130 135 140

Glu Leu Gly Tyr His Gly Val Gly Phe Gly Gly Leu Ser Val Gly Gly
 145 150 155 160

Met Val Asn Asn Arg Lys Ala Phe Ser Ala Asn Leu Leu Pro Gly Val
 165 170 175

Asp His Leu Pro His Thr Leu Asp Val Ala Arg Asn Ala Phe Thr Val
 180 185 190

Gly Leu Pro Glu His Gly Val Glu Lys Ala Glu Glu Leu Glu Arg Leu
 195 200 205

Val Thr Leu His Gly Ala Glu Asn Ile Ala Ala Val Ile Val Glu Pro
 210 215 220

Met Ser Gly Ser Ala Gly Val Val Leu Pro Pro Lys Gly Tyr Leu Gln
 225 230 235 240

Arg Leu Arg Glu Ile Thr Arg Lys His Gly Ile Leu Leu Ile Phe Asp
 245 250 255

Glu Val Ile Thr Gly Phe Gly Arg Val Gly Glu Ala Phe Ala Ala Gln
 260 265 270

Arg Trp Gly Val Val Pro Asp Leu Leu Thr Cys Ala Lys Gly Leu Thr
 275 280 285

Asn Gly Ser Ile Pro Met Gly Ala Val Phe Val Asp Glu Lys Ile His
290 295 300

Ala Ala Phe Met Gln Gly Pro Gln Gly Ala Ile Glu Phe Phe His Gly
305 310 315 320

Tyr Thr Tyr Ser Gly His Pro Val Ala Cys Ala Ala Ala Leu Ala Thr
325 330 335

Leu Asp Ile Tyr Arg Arg Asp Asp Leu Phe Gln Arg Ala Val Glu Leu
340 345 350

Glu Gly Tyr Trp Gln Asp Ala Leu Phe Ser Leu Arg Asp Leu Pro Asn
355 360 365

Val Val Asp Ile Arg Ala Val Gly Leu Val Gly Gly Val Gln Leu Ala
370 375 380

Pro His Ala Asp Gly Pro Gly Lys Arg Gly Tyr Asp Val Phe Glu Arg
385 390 395 400

Cys Phe Trp Glu His Asp Leu Met Val Arg Val Thr Gly Asp Ile Ile
405 410 415

Ala Met Ser Pro Pro Leu Ile Ile Asp Lys Pro His Ile Asp Gln Ile
420 425 430

Val Glu Arg Leu Ala Gln Ala Ile Arg Ala Ser Val
435 440

<210> 23
<211> 468
<212> PRT
<213> Pseudomonas aeruginosa PA01

<400> 23

Met Asn Ala Arg Leu His Ala Thr Ser Pro Leu Gly Asp Ala Asp Leu
1 5 10 15

Val Arg Ala Asp Gln Ala His Tyr Met His Gly Tyr His Val Phe Asp
20 25 30

Asp His Arg Val Asn Gly Ser Leu Asn Ile Ala Ala Gly Asp Gly Ala
35 40 45

Tyr Ile Tyr Asp Thr Ala Gly Asn Arg Tyr Leu Asp Ala Val Gly Gly
50 55 60

Met Trp Cys Thr Asn Ile Gly Leu Gly Arg Glu Glu Met Ala Arg Thr
 65 70 75 80

Val Ala Glu Gln Thr Arg Leu Leu Ala Tyr Ser Asn Pro Phe Cys Asp
 85 90 95

Met Ala Asn Pro Arg Ala Ile Glu Leu Cys Arg Lys Leu Ala Glu Leu
 100 105 110

Ala Pro Gly Asp Leu Asp His Val Phe Leu Thr Thr Gly Gly Ser Thr
 115 120 125

Ala Val Asp Thr Ala Ile Arg Leu Met His Tyr Tyr Gln Asn Cys Arg
 130 135 140

Gly Lys Arg Ala Lys Lys His Val Ile Thr Arg Ile Asn Ala Tyr His
 145 150 155 160

Gly Ser Thr Phe Leu Gly Met Ser Leu Gly Gly Lys Ser Ala Asp Arg
 165 170 175

Pro Ala Glu Phe Asp Phe Leu Asp Glu Arg Ile His His Leu Ala Cys
 180 185 190

Pro Tyr Tyr Tyr Arg Ala Pro Glu Gly Leu Gly Glu Ala Glu Phe Leu
 195 200 205

Asp Gly Leu Val Asp Glu Phe Glu Arg Lys Ile Leu Glu Leu Gly Ala
 210 215 220

Asp Arg Val Gly Ala Phe Ile Ser Glu Pro Val Phe Gly Ser Gly Gly
 225 230 235 240

Val Ile Val Pro Pro Ala Gly Tyr His Arg Arg Met Trp Glu Leu Cys
 245 250 255

Gln Arg Tyr Asp Val Leu Tyr Ile Ser Asp Glu Val Val Thr Ser Phe
 260 265 270

Gly Arg Leu Gly His Phe Phe Ala Ser Gln Ala Val Phe Gly Val Gln
 275 280 285

Pro Asp Ile Ile Leu Thr Ala Lys Gly Leu Thr Ser Gly Tyr Gln Pro
 290 295 300

Leu Gly Ala Cys Ile Phe Ser Arg Arg Ile Trp Glu Val Ile Ala Glu
305 310 315 320

Pro Asp Lys Gly Arg Cys Phe Ser His Gly Phe Thr Tyr Ser Gly His
325 330 335

Pro Val Ala Cys Ala Ala Ala Leu Lys Asn Ile Glu Ile Ile Glu Arg
340 345 350

Glu Gly Leu Leu Ala His Ala Asp Glu Val Gly Arg Tyr Phe Glu Glu
355 360 365

Arg Leu Gln Ser Leu Arg Asp Leu Pro Ile Val Gly Asp Val Arg Gly
370 375 380

Met Arg Phe Met Ala Cys Val Glu Phe Val Ala Asp Lys Ala Ser Lys
385 390 395 400

Ala Leu Phe Pro Glu Ser Leu Asn Ile Gly Glu Trp Val His Leu Arg
405 410 415

Ala Gln Lys Arg Gly Leu Leu Val Arg Pro Ile Val His Leu Asn Val
420 425 430

Met Ser Pro Pro Leu Ile Leu Thr Arg Glu Gln Val Asp Thr Val Val
435 440 445

Arg Val Leu Arg Glu Ser Ile Glu Glu Thr Val Glu Asp Leu Val Arg
450 455 460

Ala Gly His Arg
465

<210> 24
<211> 460
<212> PRT
<213> Pseudomonas putida W619

<400> 24

Met Asn Ala Pro Phe Gln Pro Gln Arg Asp Thr Arg Asp Tyr Gln Ala
1 5 10 15

Ser Asp Ala Ala His His Ile His Ala Phe Leu Asp Gln Lys Ala Leu
20 25 30

Asn Ala Glu Gly Pro Arg Val Ile Thr Arg Gly Glu Arg Leu Tyr Leu

35

40

45

Trp Asp Asn Asp Gly Arg Arg Tyr Leu Asp Gly Met Ser Gly Leu Trp
50 55 60

Cys Thr Gln Leu Gly Tyr Gly Arg Lys Asp Leu Thr Ala Ala Ala Ala
65 70 75 80

Ala Gln Met Asp Gln Leu Ala Tyr Tyr Asn Met Phe Phe His Thr Thr
85 90 95

His Pro Ala Val Ile Glu Leu Ser Glu Leu Leu Phe Ser Leu Leu Pro
100 105 110

Gly His Tyr Ser His Ala Ile Tyr Thr Asn Ser Gly Ser Glu Ala Asn
115 120 125

Glu Val Leu Ile Arg Thr Val Arg Arg Tyr Trp Gln Val Val Gly Gln
130 135 140

Pro Asn Lys Lys Val Met Ile Gly Arg Trp Asn Gly Tyr His Gly Ser
145 150 155 160

Thr Leu Ala Ala Thr Ala Leu Gly Gly Met Lys Phe Met His Glu Met
165 170 175

Gly Gly Leu Ile Pro Asp Val Ala His Ile Asp Glu Pro Tyr Trp Tyr
180 185 190

Ala Glu Gly Gly Glu Leu Thr Pro Ala Glu Phe Gly Arg Arg Cys Ala
195 200 205

Leu Gln Leu Glu Glu Lys Ile Leu Glu Leu Gly Ala Glu Asn Val Ala
210 215 220

Gly Phe Val Ala Glu Pro Phe Gln Gly Ala Gly Gly Met Ile Phe Pro
225 230 235 240

Pro Glu Ser Tyr Trp Pro Glu Ile Gln Arg Ile Cys Arg Gln Tyr Asp
245 250 255

Val Leu Leu Cys Ala Asp Glu Val Ile Gly Gly Phe Gly Arg Thr Gly
260 265 270

Glu Trp Phe Ala His Glu Tyr Phe Gly Phe Glu Pro Asp Thr Leu Ser
275 280 285

Ile Ala Lys Gly Leu Thr Ser Gly Tyr Val Pro Met Gly Gly Leu Val
 290 295 300

Leu Ser Lys Arg Ile Ala Glu Ala Leu Val Glu Arg Gly Gly Val Phe
 305 310 315 320

Ala His Gly Leu Thr Tyr Ser Gly His Pro Val Ala Ala Ala Val Ala
 325 330 335

Ile Ala Asn Leu Lys Ala Leu Arg Asp Glu Gly Ile Val Arg Gln Val
 340 345 350

Lys Asp Asp Thr Gly Pro Tyr Leu Gln Arg Ile Leu Arg Glu Val Phe
 355 360 365

Ala Asn His Pro Leu Ile Gly Gln Val Gln Gly Ala Gly Leu Val Ala
 370 375 380

Ala Leu Gln Phe Ala Glu Asp Lys Gly Ser Arg Lys Arg Tyr Ala Asn
 385 390 395 400

Glu Asn Asp Leu Ala Trp Gln Cys Arg Thr Tyr Gly Phe Glu Glu Gly
 405 410 415

Val Ile Ile Arg Ser Thr Leu Gly Arg Met Ile Met Ala Pro Ala Leu
 420 425 430

Val Ala Thr His Gly Glu Leu Asp Glu Leu Val Asp Lys Thr Arg Ile
 435 440 445

Ala Val Asp Arg Thr Ala Arg Gly Leu Gly Ile Leu
 450 455 460

<210> 25

<211> 459

<212> PRT

<213> Pseudomonas putida KT2440

<400> 25

Met Ser Thr His Ser Ser Thr Val Gln Asn Asp Leu Ala Ala Leu Ile
 1 5 10 15

His Pro Asn Thr Asn Leu Ala Gln His Arg Glu Val Gly Pro Leu Val
 20 30

Ile Ala Arg Gly Asp Gly Val Arg Val Phe Asp Glu Gln Gly Asn Ala
 35 40 45

Tyr Ile Glu Ala Met Ser Gly Leu Trp Ser Ala Ala Leu Gly Phe Ser
 50 55 60

Glu Gln Arg Leu Val Asp Ala Ala Val Glu Gln Phe Lys Gln Leu Pro
 65 70 75 80

Tyr Tyr His Ser Phe Ser His Lys Thr Asn Ala Pro Ala Ala Ala Leu
 85 90 95

Ala Ala Lys Leu Ala Ala Leu Ala Pro Gly Asp Leu Asn His Val Phe
 100 105 110

Phe Thr Asn Ser Gly Ser Glu Ala Asn Asp Ser Val Val Lys Met Val
 115 120 125

Trp Tyr Val Asn Asn Ala Leu Gly Arg Pro Ala Lys Lys Lys Phe Ile
 130 135 140

Ser Arg Gln Gln Ala Tyr His Gly Ala Thr Val Ala Ala Ala Ser Leu
 145 150 155 160

Thr Gly Ile Pro Ser Met His Arg Asp Phe Asp Leu Pro Ala Ile Pro
 165 170 175

Val His His Leu Thr Cys Pro Asn Phe Tyr Arg Phe Ala Arg Pro Gly
 180 185 190

Glu Ser Gln Glu Ala Phe Thr Val Arg Leu Ala Asn Glu Leu Glu Arg
 195 200 205

Tyr Ile Leu Ala Glu Gly Pro Glu Thr Ile Ala Ala Phe Ile Gly Glu
 210 215 220

Pro Val Ile Ala Ala Gly Gly Val Ile Pro Pro Pro Thr Gly Tyr Trp
 225 230 235 240

Ala Ala Ile Gln Ala Val Cys Lys Arg Tyr Asp Ile Leu Val Val Ile
 245 250 255

Asp Glu Ile Ile Thr Gly Phe Gly Arg Leu Gly Thr Met Phe Gly Ser
 260 265 270

Gln Leu Tyr Gly Ile Gln Pro Asp Ile Met Val Leu Ser Lys Gln Leu

275

280

285

Thr Ser Ser Tyr Gln Pro Leu Ala Ala Val Val Val Ser Asp Ala Met
290 295 300

Asn Asp Val Leu Val Ser Gln Ser Gln Arg Leu Gly Ala Phe Ala His
305 310 315 320

Gly Leu Thr Cys Thr Gly His Pro Val Ala Thr Ala Val Ala Leu Glu
325 330 335

Asn Ile Arg Ile Ile Glu Glu Arg Asp Leu Val Gly His Val Gln His
340 345 350

Leu Ala Pro Val Phe Gln Arg His Leu Arg Ala Phe Glu Asp His Pro
355 360 365

Leu Val Gly Asn Val Arg Gly Val Gly Leu Met Gly Gly Ile Glu Leu
370 375 380

Val Ala Asp Lys Ala Thr Arg Gln Pro Phe Ala Gln Pro Gly Thr Leu
385 390 395 400

Gly Gly Tyr Val Phe Lys Gln Ala His Lys His Gly Leu Ile Ile Arg
405 410 415

Ala Ile Tyr Asp Thr Ile Ala Phe Cys Pro Pro Leu Ile Thr Thr Gln
420 425 430

Asp Asp Ile Glu Ala Ile Phe Ser Ala Phe Glu Arg Thr Leu Ala Asp
435 440 445

Ala Thr Asp Trp Ala Arg Ser Gln His Leu Leu
450 455

<210> 26

<211> 490

<212> PRT

<213> Pseudomonas putida W619

<400> 26

Met Arg Ile Ser Ser Val Ser Ser Ala Pro Gly Ser Val Ser Ser Cys
1 5 10 15

Cys Leu Leu Cys Asp Gln Pro Leu Arg Pro Pro Ala Gly Gly Leu Trp
20 25 30

Thr Leu Glu Lys His Met Ser Val Lys Asn Pro Gln Thr Arg Asp Trp
 35 40 45

Gln Thr Leu Ser Gly Glu His His Leu Ala Pro Phe Ser Asp Tyr Lys
 50 55 60

Gln Leu Lys Glu Lys Gly Pro Arg Ile Ile Thr Lys Ala Gln Gly Val
 65 70 75 80

His Leu Trp Asp Ser Glu Gly His Lys Ile Leu Asp Gly Met Ala Gly
 85 90 95

Leu Trp Cys Val Ala Val Gly Tyr Gly Arg Glu Glu Leu Val Gln Ala
 100 105 110

Ala Glu Lys Gln Met Arg Glu Leu Pro Tyr Tyr Asn Leu Phe Phe Gln
 115 120 125

Thr Ala His Pro Pro Ala Leu Glu Leu Ala Lys Ala Ile Thr Asp Val
 130 135 140

Ala Pro Glu Gly Met Thr His Val Phe Phe Thr Gly Ser Gly Ser Glu
 145 150 155 160

Gly Asn Asp Thr Val Leu Arg Met Val Arg His Tyr Trp Ala Leu Lys
 165 170 175

Gly Lys Pro Gln Lys Gln Thr Ile Ile Gly Arg Ile Asn Gly Tyr His
 180 185 190

Gly Ser Thr Val Ala Gly Ala Ser Leu Gly Gly Met Ser Gly Met His
 195 200 205

Glu Gln Gly Gly Leu Pro Ile Pro Gly Ile Val His Ile Pro Gln Pro
 210 215 220

Tyr Trp Phe Gly Glu Gly Gly Asp Met Ser Pro Asp Asp Phe Gly Val
 225 230 235 240

Trp Ala Ala Glu Gln Leu Glu Lys Lys Ile Leu Glu Val Gly Glu Asp
 245 250 255

Asn Val Ala Ala Phe Ile Ala Glu Pro Ile Gln Gly Ala Gly Gly Val
 260 265 270

Ile Ile Pro Pro Glu Thr Tyr Trp Pro Lys Val Lys Glu Ile Leu Ala
 275 280 285

Lys Tyr Asp Ile Leu Phe Val Ala Asp Glu Val Ile Cys Gly Phe Gly
 290 295 300

Arg Thr Gly Glu Trp Phe Gly Ser Asp Tyr Tyr Asp Leu Lys Pro Asp
 305 310 315 320

Leu Met Thr Ile Ala Lys Gly Leu Thr Ser Gly Tyr Ile Pro Met Gly
 325 330 335

Gly Val Ile Val Arg Asp Lys Val Ala Lys Val Leu Ser Glu Gly Gly
 340 345 350

Asp Phe Asn His Gly Phe Thr Tyr Ser Gly His Pro Val Ala Ala Ala
 355 360 365

Val Gly Leu Glu Asn Leu Arg Ile Leu Arg Glu Glu Lys Ile Val Glu
 370 375 380

Lys Val Arg Thr Glu Val Ala Pro Tyr Leu Gln Lys Arg Leu Arg Glu
 385 390 395 400

Leu Gln Asp His Pro Leu Val Gly Glu Val Arg Gly Leu Gly Leu Leu
 405 410 415

Gly Ala Ile Glu Leu Val Lys Asp Lys Ala Ser Arg Ser Arg Tyr Glu
 420 425 430

Gly Lys Gly Val Gly Met Val Cys Arg Asn Phe Cys Phe Asp Asn Gly
 435 440 445

Leu Ile Met Arg Ala Val Gly Asp Thr Met Ile Ile Ala Pro Pro Leu
 450 455 460

Val Ile Ser His Ala Glu Val Asp Glu Leu Val Glu Lys Ala Arg Lys
 465 470 475 480

Cys Leu Asp Leu Thr Leu Glu Ala Ile Arg
 485 490

<210> 27
 <211> 457
 <212> PRT
 <213> Streptomyces coelicolor A3(2)

<400> 27

Met Ser Thr Asp Ser Pro Lys Asp Leu Ser Arg Thr Ala Tyr Asp His
1 5 10 15

Leu Trp Met His Phe Thr Arg Met Ser Ser Tyr Glu Asn Ala Pro Val
20 25 30

Pro Thr Ile Val Arg Gly Glu Gly Thr His Ile Tyr Asp Asp Lys Gly
35 40 45

Arg Arg Tyr Leu Asp Gly Leu Ala Gly Leu Phe Val Val Gln Ala Gly
50 55 60

His Gly Arg Gln Glu Leu Ala Glu Thr Ala Ser Lys Gln Ala Gln Glu
65 70 75 80

Leu Ala Phe Phe Pro Val Trp Ser Tyr Ala His Pro Lys Ala Val Glu
85 90 95

Leu Ala Glu Arg Leu Ala Asn Glu Ala Pro Gly Asp Leu Asn Lys Val
100 105 110

Phe Phe Thr Thr Gly Gly Gly Glu Ala Val Glu Thr Ala Trp Lys Leu
115 120 125

Ala Lys Gln Tyr Phe Lys Leu Thr Gly Lys Pro Thr Lys Tyr Lys Val
130 135 140

Ile Ser Arg Ala Val Ala Tyr His Gly Thr Pro Gln Gly Ala Leu Ser
145 150 155 160

Ile Thr Gly Leu Pro Ala Leu Lys Ala Pro Phe Glu Pro Leu Val Pro
165 170 175

Gly Ala His Lys Val Pro Asn Thr Asn Ile Tyr Arg Ala Pro Ile His
180 185 190

Gly Asp Asp Pro Glu Ala Tyr Gly Arg Trp Ala Ala Asp Gln Ile Glu
195 200 205

Gln Gln Ile Leu Phe Glu Gly Pro Glu Thr Val Ala Ala Val Phe Leu
210 215 220

Glu Pro Val Gln Asn Ala Gly Gly Cys Phe Pro Pro Pro Pro Gly Tyr
225 230 235 240

Phe Gln Arg Val Arg Glu Ile Cys Asp Gln Tyr Asp Val Leu Leu Val
 245 250 255

Ser Asp Glu Val Ile Cys Ala Phe Gly Arg Leu Gly Thr Thr Phe Ala
 260 265 270

Cys Asp Lys Phe Gly Tyr Val Pro Asp Met Ile Thr Cys Ala Lys Gly
 275 280 285

Met Thr Ser Gly Tyr Ser Pro Ile Gly Ala Cys Val Ile Ser Asp Arg
 290 295 300

Leu Ala Glu Pro Phe Tyr Lys Gly Asp Asn Thr Phe Leu His Gly Tyr
 305 310 315 320

Thr Phe Gly Gly His Pro Val Ser Ala Ala Val Gly Ile Ala Asn Leu
 325 330 335

Asp Leu Phe Glu Arg Glu Gly Leu Asn Gln His Val Leu Asp Asn Glu
 340 345 350

Gly Ala Phe Arg Ala Thr Leu Glu Lys Leu His Asp Leu Pro Ile Val
 355 360 365

Gly Asp Val Arg Gly Asn Gly Phe Phe Tyr Gly Ile Glu Leu Val Lys
 370 375 380

Asp Lys Ala Thr Lys Glu Ser Phe Asp Glu Glu Glu Thr Glu Arg Val
 385 390 395 400

Leu Tyr Gly Phe Leu Ser Lys Lys Leu Phe Glu Asn Gly Leu Tyr Cys
 405 410 415

Arg Ala Asp Asp Arg Gly Asp Pro Val Ile Gln Leu Ala Pro Pro Leu
 420 425 430

Ile Ser Asn Gln Glu Thr Phe Asp Glu Ile Glu Gln Ile Leu Arg Ala
 435 440 445

Thr Leu Thr Glu Ala Trp Thr Lys Leu
 450 455

<210> 28
 <211> 459
 <212> PRT
 <213> Streptomyces avermitilis MA 4680

<400> 28

Met Gly Asn Pro Ile Ala Val Ser Lys Asp Leu Ser Arg Thr Ala Tyr
1 5 10 15

Asp His Leu Trp Met His Phe Thr Arg Met Ser Ser Tyr Glu Asn Ala
20 25 30

Pro Val Pro Thr Ile Val Arg Gly Glu Gly Thr Tyr Ile Tyr Asp Asp
35 40 45

Lys Gly Lys Arg Tyr Leu Asp Gly Leu Ser Gly Leu Phe Val Val Gln
50 55 60

Ala Gly His Gly Arg Thr Glu Leu Ala Glu Thr Ala Phe Lys Gln Ala
65 70 75 80

Gln Glu Leu Ala Phe Phe Pro Val Trp Ser Tyr Ala His Pro Lys Ala
85 90 95

Val Glu Leu Ala Glu Arg Leu Ala Asn Tyr Ala Pro Gly Asp Leu Asn
100 105 110

Lys Val Phe Phe Thr Thr Gly Gly Gly Glu Ala Val Glu Thr Ala Trp
115 120 125

Lys Leu Ala Lys Gln Tyr Phe Lys Leu Gln Gly Lys Pro Thr Lys Tyr
130 135 140

Lys Val Ile Ser Arg Ala Val Ala Tyr His Gly Thr Pro Gln Gly Ala
145 150 155 160

Leu Ser Ile Thr Gly Leu Pro Ala Leu Lys Ala Pro Phe Glu Pro Leu
165 170 175

Val Pro Gly Ala His Lys Val Pro Asn Thr Asn Ile Tyr Arg Ala Pro
180 185 190

Leu Phe Gly Asp Asp Pro Glu Ala Phe Gly Arg Trp Ala Ala Asp Gln
195 200 205

Ile Glu Gln Gln Ile Leu Phe Glu Gly Pro Glu Thr Val Ala Ala Val
210 215 220

Phe Leu Glu Pro Val Gln Asn Ala Gly Gly Cys Phe Pro Pro Pro Pro
225 230 235 240

Gly Tyr Phe Gln Arg Val Arg Glu Ile Cys Asp Gln Tyr Asp Val Leu
 245 250 255

Leu Val Ser Asp Glu Val Ile Cys Ala Phe Gly Arg Leu Gly Thr Met
 260 265 270

Phe Ala Cys Asp Lys Phe Gly Tyr Val Pro Asp Met Ile Thr Cys Ala
 275 280 285

Lys Gly Met Thr Ser Gly Tyr Ser Pro Ile Gly Ala Cys Ile Val Ser
 290 295 300

Asp Arg Ile Ala Glu Pro Phe Tyr Lys Gly Asp Asn Thr Phe Leu His
 305 310 315 320

Gly Tyr Thr Phe Gly Gly His Pro Val Ser Ala Ala Val Gly Val Ala
 325 330 335

Asn Leu Asp Leu Phe Glu Arg Glu Gly Leu Asn Gln His Val Leu Asp
 340 345 350

Asn Glu Ser Ala Phe Leu Thr Thr Leu Gln Lys Leu His Asp Leu Pro
 355 360 365

Ile Val Gly Asp Val Arg Gly Asn Gly Phe Phe Tyr Gly Ile Glu Leu
 370 375 380

Val Lys Asp Lys Ala Thr Lys Glu Thr Phe Thr Asp Glu Glu Ser Glu
 385 390 395 400

Arg Val Leu Tyr Gly Phe Val Ser Lys Lys Leu Phe Glu Tyr Gly Leu
 405 410 415

Tyr Cys Arg Ala Asp Asp Arg Gly Asp Pro Val Ile Gln Leu Ser Pro
 420 425 430

Pro Leu Ile Ser Asn Gln Ser Thr Phe Asp Glu Ile Glu Ser Ile Ile
 435 440 445

Arg Gln Val Leu Thr Glu Ala Trp Thr Lys Leu
 450 455

<210> 29
 <211> 450
 <212> PRT

<213> Streptomyces avermitilis MA 4680

<400> 29

Met Thr Pro Gln Pro Asn Pro Gln Val Gly Ala Ala Val Lys Ala Ala
1 5 10 15

Asp Arg Ala His Val Phe His Ser Trp Ser Ala Gln Glu Leu Ile Asp
20 25 30

Pro Leu Ala Val Ala Gly Ala Glu Gly Ser Tyr Phe Trp Asp Tyr Asp
35 40 45

Gly Arg Arg Tyr Leu Asp Phe Thr Ser Gly Leu Val Phe Thr Asn Ile
50 55 60

Gly Tyr Gln His Pro Lys Val Val Ala Ala Ile Gln Glu Gln Ala Ala
65 70 75 80

Ser Leu Thr Thr Phe Ala Pro Ala Phe Ala Val Glu Ala Arg Ser Glu
85 90 95

Ala Ala Arg Leu Ile Ala Glu Arg Thr Pro Gly Asp Leu Asp Lys Ile
100 105 110

Phe Phe Thr Asn Gly Gly Ala Asp Ala Ile Glu His Ala Val Arg Met
115 120 125

Ala Arg Ile His Thr Gly Arg Pro Lys Val Leu Ser Ala Tyr Arg Ser
130 135 140

Tyr His Gly Gly Thr Gln Gln Ala Val Asn Ile Thr Gly Asp Pro Arg
145 150 155 160

Arg Trp Ala Ser Asp Ser Ala Ser Ala Gly Val Val His Phe Trp Ala
165 170 175

Pro Tyr Leu Tyr Arg Ser Arg Phe Tyr Ala Glu Thr Glu Gln Gln Glu
180 185 190

Cys Glu Arg Ala Leu Glu His Leu Glu Thr Thr Ile Ala Phe Glu Gly
195 200 205

Pro Gly Thr Ile Ala Ala Ile Val Leu Glu Thr Val Pro Gly Thr Ala
210 215 220

Gly Ile Met Val Pro Pro Pro Gly Tyr Leu Ala Gly Val Arg Glu Leu

225	230	235	240
Cys Asp Lys Tyr Gly Ile Val Phe Val Leu Asp Glu Val Met Ala Gly			
245	250	255	
Phe Gly Arg Thr Gly Glu Trp Phe Ala Ala Asp Leu Phe Asp Val Thr			
260	265	270	
Pro Asp Leu Met Thr Phe Ala Lys Gly Val Asn Ser Gly Tyr Val Pro			
275	280	285	
Leu Gly Gly Val Ala Ile Ser Gly Lys Ile Ala Glu Thr Phe Gly Lys			
290	295	300	
Arg Ala Tyr Pro Gly Gly Leu Thr Tyr Ser Gly His Pro Leu Ala Cys			
305	310	315	320
Ala Ala Ala Val Ala Thr Ile Asn Val Met Ala Glu Glu Gly Val Val			
325	330	335	
Glu Asn Ala Ala Asn Leu Gly Ala Arg Val Ile Glu Pro Gly Leu Arg			
340	345	350	
Glu Leu Ala Glu Arg His Pro Ser Val Gly Glu Val Arg Gly Val Gly			
355	360	365	
Met Phe Trp Ala Leu Glu Leu Val Lys Asp Arg Glu Thr Arg Glu Pro			
370	375	380	
Leu Val Pro Tyr Asn Ala Ala Gly Glu Ala Asn Ala Pro Met Ala Ala			
385	390	395	400
Phe Gly Ala Ala Ala Lys Ala Asn Gly Leu Trp Pro Phe Ile Asn Met			
405	410	415	
Asn Arg Thr His Val Val Pro Pro Cys Asn Val Thr Glu Ala Glu Ala			
420	425	430	
Lys Glu Gly Leu Ala Ala Leu Asp Ala Ala Leu Ser Val Ala Asp Glu			
435	440	445	
Tyr Thr			
450			

<210> 30
 <211> 446

<212> PRT
<213> Chromobacterium violaceum ATCC 12472

<400> 30

Met Asn His Pro Leu Thr Thr Arg Ser Glu Phe Asp His Leu Asp Leu
1 5 10 15

Arg Ala His Trp Met Pro Phe Ser Ala Asn Arg Asn Phe Gln Arg Asp
20 25 30

Pro Arg Leu Ile Val Ser Gly Glu Gly Asn Tyr Leu Thr Asp Ala Asp
35 40 45

Gly Arg Arg Ile Phe Asp Ser Leu Ser Gly Leu Trp Cys Cys Gly Ala
50 55 60

Gly His Ser Arg Lys Glu Ile Ala Glu Ala Ala Tyr Arg Gln Leu Ser
65 70 75 80

Thr Leu Asp Tyr Ser Pro Gly Phe Gln Phe Gly His Pro Leu Ser Phe
85 90 95

Arg Leu Ala Glu Arg Val Ala Ala Met Ala Pro Gly Ala Leu Asn His
100 105 110

Val Phe Phe Thr Asn Ser Gly Ser Glu Cys Ala Asp Thr Ala Val Lys
115 120 125

Met Ala Arg Ala Tyr Trp Arg Leu Lys Gly Gln Ala Ser Lys Thr Lys
130 135 140

Leu Ile Gly Arg Ala Arg Gly Tyr His Gly Val Asn Ile Ala Gly Thr
145 150 155 160

Ser Leu Gly Gly Met Asn Gly Asn Arg Lys Leu Phe Gly Pro Leu Met
165 170 175

Asp Ala Asp His Leu Pro His Thr Leu Leu Pro Ala Asn Ala Phe Ser
180 185 190

Arg Gly Leu Pro Glu Gln Gly Ala Glu Leu Ala Asp Asp Leu Leu Arg
195 200 205

Leu Ile Glu Leu His Asp Ala Ser Asn Ile Ala Ala Val Ile Val Glu
210 215 220

<220>

<223> Vector

<400> 31

gaaaaccgcc actgcgcgt taccaccgt gcgttcggtc aaggttctgg accagttgcg	60
tgagcgcata cgctacttgc attacagttt acgaaccgaa caggcttatg tcaattcgcc	120
tctcaggcgc cgctgggtgcc gctgggttga cgccaagggg gaatccgcct cgataccctg	180
attactcgct tcctgcgccc tctcaggcgg cgatagggga ctggtaaaac ggggattgcc	240
cagacgcctc ccccgcccct tcaggggcac aaatgcggcc ccaacggggc cacgtagtgg	300
tgcgtttttt gcgtttccac ctttttcttc cttttccctt ttaaaccctt taggacgtct	360
acaggccacg taatccgtgg cctgtagagt ttaaaaaggg acggatttgt tgccattaag	420
ggacggattt gttgttaaga agggacggat ttgttgttgt aaagggacgg atttgttgta	480
ttgtgggacg cagatacagt gtccccttat acacaaggaa tgtcgaacgt ggcctcacc	540
ccaatggttt acaaaaagcaa tgccctggtc gaggcgcgt atcgccctag tgttcaggaa	600
cagcggatcg ttctggcctg tattagccag gtgaagagga gcgagcctgt caccgatgaa	660
gtgatgtatt cagtgcggc ggaggacata gcgacgatgg cgggtgtccc tatcgaatct	720
tcctacaacc agctcaaaga agcggccctg cgctgaaac ggcgggaagt ccggttaacc	780
caagagccca atggcaaggg gaaaagaccg agtgtgatga ttaccggctg ggtgcaaaca	840
atcatctacc gggaggggtga gggcgtgta gaactcaggt tcaccaaaga catgctgccg	900
tacctgacgg aactcaccaa acagttcacc aaatacgctt tggctgacgt ggccaagatg	960
gacagcacc acgcgatcag gctttacgag ctgctcatgc aatgggacag catcggccag	1020
cgcgaaatag aaattgacca gctgcgaaag tggtttcaac tggaaggccg gtatccctcg	1080
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gatcttgtga ccttagctaa ggcactgtct tcagcatata tgccgattgg agccattctt	1020
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cagcacaaga agtga	1515

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 <211> 1362
 <212> DNA
 <213> Pseudomonas sp.

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cagggggtgc atttgtggga cagcgagggg cacaagatcc tcgacggcat ggcgggcctg	180
tgggtcggtg cggtgggtta cggccgtgaa gagctggttc aggcagcaga aaagcagatg	240
cgcgagctgc cgtactacaa cctgttcttc cagacggccc accgcctgc actggaactg	300
gccaaaggca ttaccgatgt ggcgcccgag ggcatgacct atgtgttctt caccggctcc	360
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<210> 41
 <211> 1359
 <212> DNA
 <213> Pseudomonas sp.

<400> 41	
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gtctacctgt gggacagcga aggccacaag atcctcgacg ccatggccgg gctctgggtgc	180
gtcaacctgg gctatggccg cgaggagctg gtcgaggccg cgaccaggca gatgcgcgag	240
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<210> 42
 <211> 1380
 <212> DNA
 <213> Artificial

<220>
 <223> codon optimized gene for expression in E coli

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 <211> 5143
 <212> DNA
 <213> Artificial

<220>
 <223> Vector

<400> 43	
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<210> 44
<211> 36
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 44
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<210> 45
<211> 37
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 45
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<210> 46
<211> 38
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 46
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<210> 47
<211> 35
<212> DNA
<213> Artificial

<220>
<223> Primer

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