MUTANT ALKB GENE

FIELD OF THE INVENTION

The present invention relates to a biotechnological means for producing at least one omega-hydroxy carboxylic acid and/or ester thereof from at least one C_6-C_{14} carboxylic acid. In particular, the biotechnological means may use a mutant AlkB gene comprising at least one specific point mutation that may be capable of producing omega-hydroxy carboxylic acid and/or ester thereof specifically.

BACKGROUND OF THE INVENTION

Omega-hydroxylated (ω-hydroxy) carboxylic acid derivatives have many commercial uses as components of industrial agents. Some examples of ω-hydroxy carboxylic acid derivatives that are useful in the industrial world include ω-hydroxy carboxylic acids; ω-hydroxy carboxylic acid methyl esters; ω-oxo carboxylic acids; ω-amino carboxylic acids; ω-amino carboxylic acid methyl esters; alpha-, omega-diacids (α, ω-diacids); omega-carboxy carboxylic acid methyl esters (ω-carboxy carboxylic acid methyl ester); alpha-, omega-diesters (α, ω-diesters); alpha-, omega-diols (α, ω-diols); and the like. These compounds may be also be used as precursors for the production of other various compounds. For example, these precursors may be used for the production of α, ω-dicarboxylic acids, and α, ω-bifunctional molecules are especially important chemicals in industrial applications for polymer resins, metal working fluids, adhesives, corrosion inhibitors, capacitor electrolytes, diester synthetic lubricants, fibres, powder coating curatives, plasticizers, polyester coatings, epoxy resins, polyamide resins, flavours, fragrances, surfactants, detergents, additives, and the like.

Generally, ω-hydroxy carboxylic acid derivatives are mainly produced from petroleum-based materials or from bioconversion of paraffin and carboxylic acids to ω-hydroxy carboxylic acid and/or derivatives thereof. The chemical methods for producing these compounds require the use of hazardous reagents. The methods used in the art are also energy intensive and cause much damage to the environment. The alternative emerging fermentation routes, while considered green processes, are still too expensive and are limited in the types and amounts of products that can be made.

Accordingly, there is a need in the art for improved green methods of producing ω-hydroxy carboxylic acid and derivatives thereof.

In one example of producing omega-amino lauric methyl ester, the precursor may be lauric acid methyl ester (LAME) where the intermediate may be omega-hydroxylauric acid methylester (HLAME). The monooxygenase AlkB is an enzyme known to catalyse the reaction forming HLAME

from LAME. The AlkB enzyme is also able to catalyse the oxidation of HLAME to omega-oxolauric acid methylester (OLAME). The monooxygenase AlkB is thus capable of catalysing both the oxidation steps resulting in the formation of OLAME from LAME via HLAME. However, AlkB enzyme is further capable of catalysing the conversion of OLAME to dodecanedioic acid monomethylester (DDAME), which results in the reduced product yield of OLAME.
There is thus a need in the art for an improved biotechnological method that increases the yield of specific omega-hydroxylated carboxylic acids and/or esters thereof from the corresponding carboxylic acids and/or esters thereof used as substrate with reduced production of unnecessary by-products. There is also a need in the art to increase the yield of the desired products such as HLAME and/or OLAME effectively.

DESCRIPTION OF THE INVENTION

The present invention attempts to solve the problems above by providing a mutant AlkB enzyme comprising at least one point mutation relative to the wild type enzyme. The mutant enzyme may be transcribed in at least one cell to produce a microbial cell expressing the mutant AlkB enzyme. This cell expressing the mutant AlkB enzyme may be capable of producing at least one omega-hydroxylated carboxylic acid and/or ester thereof from the corresponding carboxylic acid and/or ester thereof used as substrate. In particular, the carboxylic acid and/or ester thereof may be selected from the group consisting of C_6-C_{14} carboxylic acids and/or esters thereof. In particular, this cell expressing the mutant AlkB enzyme may be capable of producing more omega-hydroxylated carboxylic acids and/or esters thereof relative to a cell expressing the wild type AlkB enzyme. The yield of omega-hydroxylated carboxylic acids and/or esters thereof from the cell expressing the mutant AlkB enzyme is greater relative to the cell expressing the wild type AlkB enzyme. In one example, the mutant AlkB may be capable of increased omega-hydroxylauric acid methyl ester (HLAME) production and/or accumulation in the cell where the mutant AlkB is expressed relative to a wild type cell (i.e. where the mutant AlkB enzyme is not expressed).

According to one aspect of the present invention, there is provided at least one microbial cell expressing a mutant AlkB enzyme, the mutant AlkB enzyme comprising at least one point mutation in the wild type sequence of AlkB, wherein the point mutation is at amino acid position V129 and/or T136 of the wild type AlkB enzyme. In particular, the point mutation in the mutant AlkB enzyme is at amino acid position V129 and/or T136 of the wild type AlkB enzyme.

The archetype of this class of oxidoreductases, AlkB, is a redox protein from the *Pseudomonas putida* AlkBGT system, dependent on two auxiliary polypeptides, AlkG and AlkT. AlkT is a FAD-dependent rubredoxin reductase transferring electrons from NADH to AlkG. AlkG is a rubredoxin, an iron-containing redox protein functioning as a direct electron donor to AlkB. In an example, the term “AlkB-type oxidoreductases”, as used herein, refers to a rubredoxin-dependent oxidoreductase having a hydrocarbon binding site. In particular, the term “hydrocarbon binding site”, as used herein, refers to a stretch of the protein surface capable of binding to a hydrocarbon,

particularly an alkane and/or alkene, more particularly within reach of the catalytic centre of the protein. In one example, the term “rubredoxin-dependent alkane oxidase”, as used herein refers to an oxidoreductase that recognises as its substrate an alkane receiving electrons via a rubredoxin, the latter having, in a particular example, an iron-sulphur protein having an α+β class fold with 2 α helices and 2 to 3 β-strands transferring electrons to the alkane oxidase. More in particular, the alkB-type oxidoreductase may be AlkB from *Pseudomonas putida* Gpo1 (Access code: CAB54050.1, any access code used in the application refers to the respective sequence from the Genbank database run by the NCBI, wherein the release referred to is the one available online on the 15 Jan. 2012) or a variant thereof.

In particular, the wild type AlkB may comprise a polypeptide with sequence identity of at least 50, 60, 65, 70, 75, 80, 85, 90, 91, 94, 95, 98 or 100% to a polypeptide SEQ ID NO: 1. More in particular, the wild type AlkB has a polypeptide sequence that comprises the amino acids of SEQ ID NO:1 that are essential for the function, for example the catalytic activity of a protein, or the fold or structure of the protein. The other amino acids may be deleted, substituted or replaced by insertions or essential amino acids are replaced in a conservative manner to the effect that the biological activity of the wild type AlkB is preserved. In particular, the wild type AlkB according to any aspect of the present invention may comprise 70% sequence identity to SEQ ID NO: 1 wherein the wild type AlkB enzyme does not comprise a mutation at amino acid positions 129, 132 and 136. In particular, the wild type AlkB enzyme comprises amino acid valine (V) at amino acid position 129, leucine (L) at amino acid position 132 and threonine (T) at amino acid position 136.

The term “enzyme” means any substance composed wholly or largely of protein or polypeptides that catalyzes or promotes, more or less specifically, one or more chemical or biochemical reactions. An example of an enzyme used according to any aspect of the present invention may be AlkB. In particular, AlkB alkane hydroxylase or an alkane monooxygenase of EC 1.14.15.3. The AlkB alkane hydroxylase is a component of a reaction system comprising three enzyme components AlkB alkane hydroxylase of EC 1.14.15.3, AlkT rubredoxin NAD(P)+ reductase of EC 1.18.1.1 or of EC 1.18.1.4 and rubredoxin AlkG. AlkT is a FAD-dependent rubredoxin reductase transferring electrons from NADH to AlkG. AlkG is a rubredoxin, an iron-containing redox protein functioning as a direct electron donor to AlkB. In a preferred embodiment, the term "AlkB-type oxidoreductases", as used herein, refers to a rubredoxin-dependent oxidoreductase having a hydrocarbon binding site. The AlkB enzyme may comprise sequence identity of at least 50% to the alkane monooxygenase from *Pseudomonas putida* GPo1 encoded by AlkBGT. In particular, the AlkB wild type enzyme may comprise sequence identity of at least 70% to the polypeptide CAB54050.1 (SEQ ID NO:1; any access code used in the application refers to the respective sequence from the Genbank database run by the NCBI, wherein the release referred to is the one available online on the 28th April 2016).

A “polypeptide” (one or more peptides) is a chain of chemical building blocks called amino acids that are linked together by chemical bonds called peptide bonds. A protein or polypeptide, including an enzyme, may be “native” or “wild-type”, meaning that it occurs in nature or has the amino acid sequence of a native protein, respectively. These terms are sometimes used interchangeably. A polypeptide may or may not be glycosylated. The term “wild type” as used herein in conjunction with a cell or microorganism may denote a cell with a genome make-up that is in a form as seen naturally in the wild. The term may be applicable for both the whole cell and for individual genes. The term ‘wild type’ may thus also include cells which have been genetically modified in other aspects (i.e. with regard to one or more genes) but not in relation to the genes of interest. The term “wild type” therefore does not include such cells where the gene sequences of the specific genes of interest have been altered at least partially by man using recombinant methods. A wild type cell according to any aspect of the present invention thus refers to a cell that has no genetic mutation with respect to the whole genome and/or a particular gene. Therefore, in one example, a wild type AlkB may be an enzyme that is found naturally in nature without any of the specific mutations according to any aspect of the present invention. In another example, the wild type cell relative to the mutant AlkB according to any aspect of the present invention relates to a cell that has the natural/ non-altered expression of AlkB with mutations that may occur naturally but not at amino acid positions 129, 132 and 136.

A polypeptide may also be a “mutant” meaning that it has been made, altered, derived, or is in some way different or changed from its wild-type form, or from another mutant. Mutant proteins typically have amino acid substitutions at one or more positions. Mutant DNA molecules typically have nucleotide substitutions in one or more positions. Mutant forms of a protein or DNA molecule can have the same, or altered, functions in comparison to the wild-type. For simplification, mutants may be referred to by their variation from the single amino acid code from which the mutation arose. For example, in one format the mutant is referred to as XPOSY, where “X” refers to the single letter code of the amino acid in the original sequence, “POS” refers to the position of the mutation in the sequence, and Y refers to the single letter code for the new amino acid appearing at the mutation's position. For example, V129T would mean that in the original protein, the amino acid at position 129 is a valine (“V”), but in the mutant, the valine is replaced with a threonine (“T”).

In particular, the mutant AlkB enzyme according to any aspect of the present invention may comprise at least one point mutation at amino acid positions 129, 132 and/or 136 of the AlkB enzyme. In particular, the point mutations may be at amino acid positions 129, 132 or 136. In another example, the point mutation may be at amino acid position 129 and 132 or 129 and 136 or 132 and 136. In a further example, the point mutation may be at amino acid positions 129, 136 and 136. More in particular, the amino acid valine at position 129 may be converted to an amino acid selected from the group consisting of glycine, alanine, leucine, isoleucine, proline, phenylalanine, tyrosine, tryptophan, serine, threonine, cysteine, methionine, asparagine, glutamine, lysine, arginine, histidine, aspartate and glutamate. In particular, the amino acid valine at position 129 may

be converted to an amino acid selected from the group consisting of threonine, alanine, serine, cysteine, asparagine and glycine. More in particular, the amino acid valine at position 129 may be converted to threonine or alanine. The amino acid threonine at position 136 may also be converted to an amino acid selected from the group consisting of glycine, alanine, leucine, isoleucine, proline, phenylalanine, tyrosine, tryptophan, serine, valine, cysteine, methionine, asparagine, glutamine, lysine, arginine, histidine, aspartate and glutamate. In particular, the amino acid threonine at position 136 may be converted to an amino acid selected from the group consisting of glycine, alanine, leucine, isoleucine, proline, phenylalanine, tryptophan, serine, valine, cysteine and methionine. The point mutations according to any aspect of the present invention may be position 129 and/or 136. In one example, the point mutation in mutant AlkB according to any aspect of the present invention may be at position 129. In another example, the point mutation in mutant AlkB according to any aspect of the present invention may be at position 136. In a further example, the point mutation in mutant AlkB according to any aspect of the present invention may be at position 129 and 136 in combination. More in particular, the point mutations of the cell according to any aspect of the present invention may be selected from the group consisting of V129T, T136A, V129A and combinations thereof relative to the wild type sequence of AlkB. The mutant AlkB enzyme may comprise V129T, T136A or V129A point mutation. In particular, the AlkB enzyme according to any aspect of the present invention may comprise the point mutation V129T. In one example, the AlkB enzyme may further comprise the point mutation T136A. In another example, the mutant AlkB enzyme of any cell according to any aspect of the present invention may comprise the point mutations V129T and T136A, V129A and T136A.

In particular, a microbial cell expressing a mutant AlkB enzyme, the mutant AlkB enzyme comprising at least one point mutation in the wild type sequence of AlkB, wherein the point mutation is at amino acid position V129 and/or T136 of the wild type AlkB enzyme wherein the point mutation at amino acid V129 is selected from the group consisting of V129T and V129A. More in particular, the point mutation may be at amino acid position T136. Even more in particular, the point mutation at amino acid position T136 may be T136A. In one example, when the point mutation is at amino acid position V129, the mutation may be selected from the group consisting of V129T and V129A. In another example, the point mutation may be at amino acid positions V129 and T136. In particular. the point mutation at amino acid position V129 may be selected from the group consisting of V129T and V129A and the point mutation at amino acid position T136 is T136A.

In particular, the point mutation may be V129A. In one example, the wild type sequence may be SEQ ID NO:1 or a variant thereof.

A "variant" of AlkB or at least SEQ ID NO:1, as used herein, refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties (e.g., replacement of leucine

with isoleucine). More rarely, a variant may have "non-conservative" changes (e.g., replacement of glycine with tryptophan). Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software. According to any aspect of the present invention, the variant of SEQ ID NO:1 used as the wild type AlkB may not comprise a mutation at amino acid positions 129, 132 and/or 136.

Any techniques in molecular biology can be used for producing AlkB enzymes with specific point mutations. The term 'point mutation', or single base modification, is a type of mutation that causes a single nucleotide base change, insertion, or deletion of the genetic material, DNA or RNA. In one example, point mutation may refer to an amino acid substitution in a wild type sequence of AlkB gene. Some examples of method that may be used include error-prone polymerase chain reaction, cassette mutagenesis (in which the specific region optimized is replaced with a synthetically mutagenized oligonucleotide), oligonucleotide-directed mutagenesis, parallel PCR (which uses a large number of different PCR reactions that occur in parallel in the same vessel, such that the product of one reaction primes the product of another reaction), random mutagenesis (e.g., by random fragmentation and reassembly of the fragments by mutual priming); site-specific mutations (introduced into long sequences by random fragmentation of the template followed by reassembly of the fragments in the presence of mutagenic oligonucleotides); parallel PCR (e.g., recombination on a pool of DNA sequences); sexual PCR; and chemical mutagenesis (e.g., by sodium bisulfite, nitrous acid, hydroxylamine, hydrazine, formic acid, or by adding nitrosoguanidine, 5-bromouracil, 2-aminopurine, and acridine to the PCR reaction in place of the nucleotide precursor; or by adding intercalating agents such as proflavine, acriflavine, quinacrine); irradiation (X-rays or ultraviolet light, and/or subjecting the polynucleotide to propagation in a host cell that is deficient in normal DNA damage repair function); or DNA shuffling (e.g., in vitro or in vivo homologous recombination of pools of nucleic acid fragments or polynucleotides). Any one of these techniques can also be employed under low-fidelity polymerization conditions to introduce a low level of point mutations randomly over a long sequence, or to mutagenize a mixture of fragments of unknown sequence.

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

According to any aspect of the present invention, the microbial cell may be selected from the species of bacteria from the group consisting of, *Abiotrophia, Acaryochloris, Accumulibacter, Acetivibrio, Acetobacter, Acetohalobium, Acetonema, Achromobacter, Acidaminococcus, Acidimicrobium, Acidiphilium, Acidithiobacillus, Acidobacterium, Acidothermus, Acidovorax, Acinetobacter, Actinobacillus, Actinomyces, Actinosynnema, Aerococcus, Aeromicrobium, Aeromonas, Afipia, Aggregatibacter, Agrobacterium, Ahrensia, Akkermansia, Alcanivorax, Alicycliphilus, Alicyclobacillus, Aliivibrio, Alkalilimnicola, Alkaliphilus, Allochromatium, Alteromonadales, Alteromonas, Aminobacterium, Aminomonas, Ammonifex, Amycolatopsis, Amycolicicoccus, Anabaena, Anaerobaculum, Anaerococcus, Anaerofustis, Anaerolinea, Anaeromyxobacter, Anaerostipes, Anaerotruncus, Anaplasma, Anoxybacillus, Aquifex, Arcanobacterium, Arcobacter, Aromatoleum, Arthrobacter, Arthrospira, Asticcacaulis, Atopobium, Aurantimonas, Azoarcus, Azorhizobium, Azospirillum, Azotobacter, Bacillus, Bartonella, Basfia, Baumannia, Bdellovibrio, Beggiatoa, Beijerinckia, Bermanella, Beutenbergia, Bifidobacterium, Bilophila, Blastopirellula, Blautia, Blochmannia, Bordetella, Borrelia, Brachybacterium, Brachyspira, Bradyrhizobium, Brevibacillus, Brevibacterium, Brevundimonas, Brucella, Buchnera, Bulleidia, Burkholderia, Butyrivibrio, Caldalkalibacillus, Caldanaerobacter, Caldicellulosiruptor, Calditerrivibrio, Caminibacter, Campylobacter, Carboxydibrachium, Carboxydothermus, Cardiobacterium, Carnobacterium, Carsonella, Catenibacterium, Catenulispora, Catonella, Caulobacter, Cellulomonas, Cellvibrio, Centipeda, Chelativorans, Chloroflexus, Chromobacterium, Chromohalobacter, Chthoniobacter, Citreicella, Citrobacter, Citromicrobium, Clavibacter, Cloacamonas, Clostridium, Collinsella, Colwellia, Comamonas, Conexibacter, Congregibacter, Coprobacillus, Coprococcus, Coprothermobacter, Coraliomargarita, Coriobacterium, corrodens, Corynebacterium, Coxiella, Crocosphaera, Cronobacter, Cryptobacterium, Cupriavidus, Cyanobium, Cyanothece, Cylindrospermopsis, Dechloromonas, Deferribacter, Dehalococcoides, Dehalogenimonas, Deinococcus, Delftia, Denitrovibrio, Dermacoccus, Desmospora, Desulfarculus, Desulphateibacillum, Desulfitobacterium, Desulfobacca, Desulfobacterium, Desulfobulbus, Desulfococcus, Desulfohalobium, Desulfomicrobium, Desulfonatronospira, Desulforudis, Desulfotalea, Desulfotomaculum, Desulfovibrio, Desulfurispirillum, Desulfurobacterium, Desulfuromonas, Dethiobacter, Dethiosulfovibrio, Dialister, Dichelobacter, Dickeya, Dictyoglomus, Dietzia, Dinoroseobacter, Dorea, Edwardsiella, Ehrlichia, Eikenella, Elusimicrobium, Endoriftia, Enhydrobacter, Enterobacter, Enterococcus, Epulopiscium, Erwinia, Erysipelothrix, Erythrobacter, Escherichia, Ethanoligenens, Eubacterium, Eubacterium, Exiguobacterium, Faecalibacterium, Ferrimonas, Fervidobacterium, Fibrobacter, Finegoldia, Flexistipes, Francisella, Frankia, Fructobacillus, Fulvimarina, Fusobacterium, Gallibacterium, Gallionella, Gardnerella, Gemella, Gemmata, Gemmatimonas, Geobacillus, Geobacter, Geodermatophilus, Glaciecola, Gloeobacter, Glossina, Gluconacetobacter, Gordonia, Granulibacter, Granulicatella, Grimontia, Haemophilus, Hahella, Halanaerobiumns, Haliangium, Halomonas, Halorhodospira, Halothermothrix, Halothiobacillus, Hamiltonella, Helicobacter, Heliobacterium, Herbaspirillum, Herminiimonas, Herpetosiphon, Hippea, Hirschia, Histophilus, Hodgkinia, Hoeflea, Holdemania, Hydrogenivirga, Hydrogenobaculum, Hylemonella, Hyphomicrobium, Hyphomonas, Idiomarina, Ilyobacter,*

Intrasporangium, Isoptericola, Isosphaera, Janibacter, Janthinobacterium, Jonesia, Jonquetella, Kangiella, Ketogulonicigenium, Kineococcus, Kingella, Klebsiella, Kocuria, Koribacter, Kosmotoga, Kribbella, Ktedonobacter, Kytococcus, Labrenzia, Lactobacillus, Lactococcus, Laribacter, Lautropia, Lawsonia, Legionella, Leifsonia, Lentisphaera, Leptolyngbya, Leptospira, Leptothrix, Leptotrichia, Leuconostoc, Liberibacter, Limnobacter, Listeria, Loktanella, Lutiella, Lyngbya, Lysinibacillus, Macrococcus, Magnetococcus, Magnetospirillum, Mahella, Mannheimia, Maricaulis, Marinithermus, Marinobacter, Marinomonas, Mariprofundus, Maritimibacter, Marvinbryantia, Megasphaera, Meiothermus, Melissococcus, Mesorhizobium, Methylacidiphilum, Methylibium, Methylobacillus, Methylobacter, Methylobacterium, Methylococcus, Methylocystis, Methylomicrobium, Methylophaga, Methylophilales, Methylosinus, Methyloversatilis, Methylovorus, Microbacterium, Micrococcus, Microcoleus, Microcystis, Microlunatus, Micromonospora, Mitsuokella, Mobiluncus, Moorella, Moraxella, Moritella, Mycobacterium, Myxococcus, Nakamurella, Natranaerobius, Neisseria, Neorickettsia, Neptuniibacter, Nitratifractor, Nitratiruptor, Nitrobacter, Nitrococcus, Nitrosomonas, Nitrosospira, Nitrospira, Nocardia, Nocardioides, Nocardiopsis, Nodularia, Nostoc, Novosphingobium, Oceanibulbus, Oceanicaulis, Oceanicola, Oceanithermus, Oceanobacillus, Ochrobactrum, Octadecabacter, Odyssella, Oligotropha, Olsenella, Opitutus, Oribacterium, Orientia, Ornithinibacillus, Oscillatoria, Oscillochloris, Oxalobacter, Paenibacillus, Pantoea, Paracoccus, Parascardovia, Parasutterella, Parvibaculum, Parvimonas, Parvularcula, Pasteurella, Pasteuria, Pectobacterium, Pediococcus, Pedosphaera, Pelagibaca, Pelagibacter, Pelobacter, Pelotomaculum, Peptoniphilus, Peptostreptococcus, Persephonella, Petrotoga, Phaeobacter, Phascolarctobacterium, Phenylobacterium, Photobacterium, Pirellula, Planctomyces, Planococcus, Plesiocystis, Polaromonas, Polaromonas, Polymorphum, Polynucleobacter, Poribacteria, Prochlorococcus, Propionibacterium, Proteus, Providencia, Pseudoalteromonas, Pseudoflavonifractor, Pseudomonas, Pseudonocardia, Pseudoramibacter, Pseudovibrio, Pseudoxanthomonas, Psychrobacter, Psychromonas, Puniceispirillum, Pusillimonas, Pyramidobacter, Rahnella, Ralstonia, Raphidiopsis, Regiella, Reinekea, Renibacterium, Rhizobium, Rhodobacter, Rhodococcus, Rhodoferax, Rhodomicrobium, Rhodopirellula, Rhodopseudomonas, Rhodospirillum, Rickettsia, Rickettsiella, Riesia, Roseburia, Roseibium, Roseiflexus, Roseobacter, Roseomonas, Roseovarius, Rothia, Rubrivivax, Rubrobacter, Ruegeria, Ruminococcus, Ruthia, Saccharomonospora, Saccharophagus, Saccharopolyspora, Sagittula, Salinispora, Salmonella, Sanguibacte, Scardovia, Sebaldella, Segniliparus, Selenomonas, Serratia, Shewanella, Shigella, Shuttleworthia, Sideroxydans, Silicibacter, Simonsiella, Sinorhizobium, Slackia, Sodalis, Solibacter, Solobacterium, Sorangium, Sphaerobacter, Sphingobium, Sphingomonas, Sphingopyxis, Spirochaeta, Sporosarcina, Stackebrandtia, Staphylococcus, Starkeya, Stenotrophomonas, Stigmatella, Streptobacillus, Streptococcus, Streptomyces, Streptosporangium, Subdoligranulum, subvibrioides, Succinatimonas, Sulfitobacter, Sulfobacillus, Sulfuricurvum, Sulfurihydrogenibium, Sulfurimonas, Sulfurospirillum, Sulfurovum, Sutterella, Symbiobacterium, Synechocystis, Syntrophobacter, Syntrophobotulus, Syntrophomonas, Syntrophothermus, Syntrophus, taiwanensis, Taylorella, Teredinibacter, Terriglobus, Thalassiobium, Thauera, Thermaerobacter, Thermanaerovibrio,

Thermincola, Thermoanaerobacter, Thermoanaerobacterium, Thermobaculum, Thermobifida, Thermobispora, Thermocrinis, Thermodesulphateator, Thermodesulfobacterium, Thermodesulfobium, Thermodesulfovibrio, Thermomicrobium, Thermomonospora, Thermosediminibacter, Thermosinus, Thermosipho, Thermosynechococcus, Thermotoga, Thermovibrio, Thermus, Thioalkalimicrobium, Thioalkalivibrio, Thiobacillus, Thiomicrospira, Thiomonas, Tolumonas, Treponema, tribocorum, Trichodesmium, Tropheryma, Truepera, Tsukamurella, Turicibacter, Variovorax, Veillonella, Verminephrobacter, Verrucomicrobium, Verrucosispora, Vesicomyosocius, Vibrio, Vibrionales, Victivallis, Weissella, Wigglesworthia, Wolbachia, Wolinella, Xanthobacter, Xanthomonas, Xenorhabdus, Xylanimonas, Xylella, Yersinia, Zinderia and Zymomonas,

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

In another example, the microbial cell may be a yeast cell. Suitable yeasts according to any aspect of the present invention may belong to the genera listed at http://www.dsmz.de/species/yeasts.htm. In particular, the yeast may be selected from the group consisting of *Schizosaccharomyces pombe*, *Schizosaccharomyces cerevisiae* and *Pichia pastoris*.

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

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

time interval in a suitable nutrient medium and then determining the amount of target product (ω-functionalized carboxylic acid ester) in the nutrient medium. The genetically modified cell or microorganism may be genetically different from the wild type cell or microorganism. The genetic difference between the genetically modified microorganism according to any aspect of the present invention and the wild type microorganism may be in the presence of a complete gene, amino acid, nucleotide etc. in the genetically modified microorganism that may be absent in the wild type microorganism. In one example, the genetically modified microorganism according to any aspect of the present invention may comprise enzymes, in particular mutant AlkB that enables the microorganism to produce more HLAME relative to the wild type cell with the wild type or no AlkB expression. The wild type microorganism relative to the genetically modified microorganism of the present invention may have none or no detectable activity of the enzymes. As used herein, the term 'genetically modified microorganism' may be used interchangeably with the term 'genetically modified cell'. The genetic modification according to any aspect of the present invention is carried out on the cell of the microorganism.

The cells according to any aspect of the present invention are genetically transformed according to any method known in the art. In particular, the cells may be produced according to the method disclosed in WO2013024114. The phrase 'the genetically modified cell has an increased activity, in comparison with its wild type, in enzymes' as used herein refers to the activity of the respective enzyme that is increased by a factor of at least 2, in particular of at least 10, more in particular of at least 100, yet more in particular of at least 1000 and even more in particular of at least 10000.

The mutant AlkB enzyme according to any aspect of the present invention may be capable of

(a) catalysing the conversion of C_6-C_{14} carboxylic acids and/or esters thereof as substrate to the corresponding omega-hydroxy carboxylic acid and/or esters thereof; or

(b) catalysing the conversion of C_6-C_{14} carboxylic acids and/or esters thereof as substrate to the corresponding omega-oxocarboxylic acids and/or esters thereof.

The term "substrate" means any substance or compound that is converted or meant to be converted into another compound by the action of an enzyme catalyst. The term includes aromatic and aliphatic compounds, and includes not only a single compound, but also combinations of compounds, such as solutions, mixtures and other materials which contain at least one substrate. According to any aspect of the present invention, the substrate may be at least one C_6-C_{14} carboxylic acid and/or esters thereof. The substrate may thus be selected from the group consisting of C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14} and esters thereof. The substrate may be a combination of C_6-C_{14} carboxylic acid and/or esters thereof. In particular, the substrate may be at least one C_8-C_{12} carboxylic acid and/or ester thereof, at least one C_6-C_{10} carboxylic acid and/or ester thereof, or at least one C_8-C_{10} carboxylic acid and/or ester thereof. In one example, the

substrate may be lauric acid and/or ester thereof. In particular, the ester may be an ethyl or methyl ester of the carboxylic acid. In one example, the ester as substrate according to any aspect of the present invention may be methyl lauric acid methyl ester (LAME). In this example, the mutant AlkB may be capable of catalysing the oxidation reaction of conversion of lauric acid and/or lauric acid methyl ester (LAME) to omega-hydroxy lauric acid and/or omega-hydroxylauric acid methyl ester (HLAME) respectively. In another example, the mutant AlkB may be capable of catalysing the conversion of lauric acid and/or lauric acid methyl ester (LAME) to omega-oxo lauric acid and/or omega-oxolauric acid methyl ester (OLAME) respectively. In yet a further example, the mutant AlkB may be capable of catalysing the conversion of lauric acid and/or lauric acid methyl ester (LAME) to omega-hydroxy lauric acid and/or omega-hydroxylauric acid methyl ester (HLAME) and omega-oxo lauric acid and/or omega-oxolauric acid methyl ester (OLAME) concurrently.

An “oxidation reaction” or “oxygenation reaction”, as used herein, is a chemical or biochemical reaction involving the addition of oxygen to a substrate, to form an oxygenated or oxidized substrate or product. An oxidation reaction is typically accompanied by a reduction reaction (hence the term “redox” reaction, for oxidation and reduction). A compound is “oxidized” when it receives oxygen or loses electrons. A compound is “reduced” when it loses oxygen or gains electrons. Some examples of oxidative reactions include but are not limited to the conversion of LAME to HLAME, HLAME to OLAME, OLAME to DDAME and the like.

In particular, the mutant AlkB enzyme may be capable of producing more omega-hydroxy- and/or omega-oxo carboxylic acids and/or esters thereof from carboxylic acids than a wild type AlkB enzyme. There will also be less by products formed.

In one example, the effect of heterologous expression of the AlkB mutants according to any aspect of the present invention may be analysed using *Escherichia coli* as the host cell. These mutant AlkB enzymes may be transformed into *E.coli* and these transformed cells grown under process relevant conditions in a bioreactor in the presence of a substrate such as LAME. Compared to any reference strain expressing wild type AlkB, the mutant strains may be shown to have a significant increase in bioconversion rate of LAME towards HLAME as well as an up to twofold improved ratio of HLAME:DDAME. This ratio may indicate a reduced substrate specificity for HLAME, since HLAME is the precursor of DDAME production in the AlkB reaction cascade. The cells according to any aspect of the present invention may thus give rise to a more energy efficient process in which the space-time-yield as well as product yield may be increased contributing to a more economical polyamide 12 precursor production process.

In particular, the cell and/or method according to any aspect of the present invention may be capable of increasing omega-hydroxylauric acid methylester (HLAME) to dodecanedioic acid monomethylester (DDAME) (HLAME/DDAME) ratio relative to the HLAME/DDAME ratio in a wild type cell. In particular, this method comprises contacting the cell according to any aspect of the

present invention with the substrate, LAME. This ratio is calculated by dividing the total amount of HLAME produced in the reaction mixture with the total amount of DDAME produced in the reaction at a given point in time. In particular, the amount of HLAME and/or DDAME may be measured at any given point in time by any known method in the art. More in particular, the concentration of HLAME and/or DDAME may be measured using gas or liquid chromatography coupled with mass spectrometry. The higher the concentration of HLAME, the higher the ratio. In particular, the HLAME/DDAME ratio may at least be 1:1. This in itself may be higher than the wild type enzyme and/or cell. More in particular, the HLAME/DDAME ratio may at least be about 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1, 1.6:1, 1.7:1, 1.8:1, 1.9:1,2:1, 2.1:1, 2.2:1, 2.3:1, 2.4:1, 2.5;1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1 and the like. Even more in particular, the HLAME/DDAME ratio may at least be 1.5:1.

In another example, the cell and/or method according to any aspect of the present invention may be capable of increasing omega-aminolauric acid methylester (ALAME) to dodecanedioic acid monomethylester (DDAME) (ALAME/DDAME) ratio relative to the ALAME/DDAME ratio in a wild type cell. In particular, this method comprises contacting the cell according to any aspect of the present invention with the substrate, LAME. This ratio is calculated by dividing the total amount of ALAME produced in the reaction mixture with the total amount of DDAME produced in the reaction at a given point in time. In particular, the amount of ALAME and/or DDAME may be measured at any given point in time by any known method in the art. More in particular, the concentration of ALAME and/or DDAME may be measured using gas or liquid chromatography coupled with mass spectrometry. The higher the concentration of ALAME, the higher the ratio. In particular, the ALAME/DDAME ratio may at least be 1:1. This in itself may be higher than the wild type enzyme and/or cell. More in particular, the ALAME/DDAME ratio may at least be about 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1, 1.6:1, 1.7:1, 1.8:1, 1.9:1,2:1, 2.1:1, 2.2:1, 2.3:1, 2.4:1, 2.5;1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1 and the like. Even more in particular, the ALAME/DDAME ratio may at least be 1.5:1.

According to a further aspect of the present invention, there is provided a use of the mutant AlkB enzyme and or cell comprising and expressing the mutant AlkB enzyme according to any aspect of the present invention for catalysing the conversion of lauric acid methyl ester (LAME) to omega-hydroxylauric acid methyl ester (HLAME).

As used herein, “about” or “approximately” shall mean within 20 percent, particularly within 10 percent, and more particularly within 5 percent of a given value or range.

In particular, the mutant AlkB enzyme according to any aspect of the present invention may have a decreased Km value or increased vmax value relative to the wild type AlkB enzyme with respect to the substrate carboxylic acids and/or esters thereof of C_6-C_{14} with non-functionalized terminal methyl bonds, such as carboxylic acid methyl, ethyl or propylesters or alkanes, thus resulting in improved activity of mutant AlkB. For example, the mutant AlkB enzyme according to any aspect of the present invention may be capable of recognizing LAME and thus catalyzing the oxidative

conversion of substrate LAME to HLAME. In another example, the mutant AlkB enzyme according to any aspect of the present invention may have an increased Km value or decreased vmax value relative to the wild type AlkB enzyme with respect to the substrate OLAME or other substrates with oxo-functionalized terminal bonds, such as omega-oxo carboxylic acid methyl, ethyl or propylesters or alkanals, thus resulting in decreased activity. In a further example, the mutant AlkB enzyme according to any aspect of the present invention may have an increased Km value or decreased vmax value relative to the wild type AlkB enzyme with respect to the substrate HLAME or other substrates with hydroxy-functionalized terminal bonds, such as omega-hydroxy fatty acid methyl, ethyl or propylesters or alkanols, thus resulting in decreased activity.

Accordingly, the mutant AlkB enzyme according to any aspect of the present invention may be useful for the production and/or accumulation of HLAME. In particular, the mutant AlkB enzyme according to any aspect of the present invention may be capable of producing more HLAME relative to the wild type AlkB enzyme and/or this HLAME may not be converted into other downstream products such as OLAME and/or DDAME. Alternatively, the mutant AlkB enzyme according to any aspect of the present invention may be useful for the production and/or accumulation of more HLAME relative to DDAME produced simultaneously as an unwanted by-product. In particular, the mutant AlkB enzyme according to any aspect of the present invention may be capable of producing more HLAME relative to DDAME which may be produced simultaneously as an unwanted by-product, in comparison to the wild type AlkB enzyme. There is thus less wasted resources as a small amount of substrate LAME may be sufficient for the production of large amounts of HLAME relative to the wild type AlkB enzyme. In particular, it could be demonstrated that the AlkB mutants according to any aspect of the present invention proved to have a higher HLAME productivity and additionally a decreased substrate specificity for HLAME and/or OLAME (resulting in an increased HLAME/DDAME product ratio) compared to wildtype AlkB. A similar explanation may be relevant for the increased ALAME/DDAME ratio that may be seen. In one example, endogenous alcohol dehydrogenases found in the cells according to any aspect of the present invention may be involved in the formation of the ALAME thus showing no increase in OLAME.

The cell according to any aspect of the present invention may be further genetically modified to comprise increased expression relative to a wild type cell of an enzyme E_1, an ω-transaminase. In particular, enzyme E_1 may be capable of catalysing the conversion of the omega-oxocarboxylic acids and/or esters thereof to the corresponding omega-aminocarboxylic acids and/or esters thereof.

The phrase "increased activity of an enzyme", as used herein is to be understood as increased intracellular activity. Basically, an increase in enzymatic activity can be achieved by increasing the copy number of the gene sequence or gene sequences that code for the enzyme, using a strong promoter or employing a gene or allele that codes for a corresponding enzyme with increased

activity, altering the codon utilization of the gene, increasing the half-life of the mRNA or of the enzyme in various ways, modifying the regulation of the expression of the gene and optionally by combining these measures. Genetically modified cells used according to any aspect of the present invention are for example produced by transformation, transduction, conjugation or a combination of these methods with a vector that contains the desired gene, an allele of this gene or parts thereof and a vector that makes expression of the gene possible. Heterologous expression is in particular achieved by integration of the gene or of the alleles in the chromosome of the cell or an extrachromosomally replicating vector. In one example, the cell according to any aspect of the present invention may comprise increased expression relative to the wild type cell of ω-transaminase (E_1). In particular, the ω-transaminase (E_1) is from EC 2.6.1. In particular, the Enzyme E_1, may be an aminotransferase (E_n) selected from the group consisting of *Pseudomonas putida* (WP_016502144; WP_016500675.1), *Chromobacterium violaceum* (NP_901695.1), *Rhodobacter sphaeroides 2.4.1*(YP_353455.1) and 3HMU_A, AAD41041.1, AAK15486.1, ABE03917.1, ADR60699.1, ADR61066.1, ADR62525.1, AEL07495.1, CAZ86955.1, EFW82310.1, EFW87681.1, EGC99983.1, EGD03176.1, EGE58369.1, EGH06681.1, EGH08331.1, EGH24301.1, EGH32343.1, EGH46412.1, EGH55033.1, EGH62152.1, EGH67339.1, EGH70821.1, EGH71404.1, EGH78772.1, EGH85312.1, EGH97105.1, EGP57596.1, NP_102850.1, NP_106560.1, NP_248912.1, NP_248990.1, NP_354026.2, NP_421926.1, NP_637699.1, NP_642792.1, NP_744329.1, NP_744732.1, NP_747283.1, NP_795039.1, XP_002943905.1, YP_001021095.1, YP_001059677.1, YP_001061726.1, YP_001066961.1, YP_001074671.1, YP_001120907.1, YP_001140117.1, YP_001170616.1, YP_001185848.1, YP_001188121.1, YP_001233688.1, YP_001268866.1, YP_001270391.1, YP_001345703.1, YP_001412573.1, YP_001417624.1, YP_001526058.1, YP_001579295.1, YP_001581170.1, YP_001668026.1, YP_001669478.1, YP_001671460.1, YP_001685569.1, YP_001747156.1, YP_001749732.1, YP_001765463.1, YP_001766294.1, YP_001790770.1, YP_001808775.1, YP_001809596.1, YP_001859758.1, YP_001888405.1, YP_001903233.1, YP_001977571.1, YP_002229759.1, YP_002231363.1, YP_002280472.1, YP_002297678.1, YP_002543874.1, YP_002549011.1, YP_002796201.1, YP_002801960.1, YP_002875335.1, YP_002897523.1, YP_002912290.1, YP_002974935.1, YP_003060891.1, YP_003264235.1, YP_003552364.1, YP_003578319.1, YP_003591946.1, YP_003607814.1, YP_003641922.1, YP_003674025.1, YP_003692877.1, YP_003755112.1, YP_003896973.1, YP_003907026.1, YP_003912421.1, YP_004086766.1, YP_004142571.1, YP_004147141.1, YP_004228105.1, YP_004278247.1, YP_004305252.1, YP_004356916.1, YP_004361407.1, YP_004378186.1, YP_004379856.1, YP_004390782.1, YP_004472442.1, YP_004590892.1, YP_004612414.1, YP_004676537.1, YP_004693233.1, YP_004701580.1, YP_004701637.1, YP_004704442.1, YP_108931.1, YP_110490.1, YP_168667.1, YP_237931.1, YP_260624.1, YP_262985.1, YP_271307.1, YP_276987.1, YP_334171.1, YP_337172.1, YP_350660.1, YP_351134.1, YP_364386.1, YP_366340.1, YP_369710.1, YP_370582.1, YP_426342.1, YP_440141.1, YP_442361.1, YP_468848.1, YP_521636.1, YP_554363.1, YP_608454.1, YP_610700.1, YP_614980.1, YP_622254.1, YP_625753.1, YP_680590.1, YP_751687.1, YP_767071.1, YP_774090.1,

YP_774932.1, YP_788372.1, YP_858562.1, YP_928515.1, YP_983084.1, YP_995622.1, ZP_00948889.1, ZP_00954344.1, ZP_00959736.1, ZP_00998881.1, ZP_01011725.1, ZP_01037109.1, ZP_01058030.1, ZP_01076707.1, ZP_01103959.1, ZP_01167926.1, ZP_01224713.1, ZP_01442907.1, ZP_01446892.1, ZP_01550953.1, ZP_01625518.1, ZP_01745731.1, ZP_01750280.1, ZP_01754305.1, ZP_01763880.1, ZP_01769626.1, ZP_01865961.1, ZP_01881393.1, ZP_01901558.1, ZP_02145337.1, ZP_02151268.1, ZP_02152332.1, ZP_02167267.1, ZP_02190082.1, ZP_02242934.1, ZP_02360937.1, ZP_02367056.1, ZP_02385477.1, ZP_02456487.1, ZP_02883670.1, ZP_03263915.1, ZP_03263990.1, ZP_03400081.1, ZP_03452573.1, ZP_03456092.1, ZP_03517291.1, ZP_03529055.1, ZP_03571515.1, ZP_03572809.1, ZP_03587785.1, ZP_03588560.1, ZP_03697266.1, ZP_03697962.1, ZP_04521092.1, ZP_04590693.1, ZP_04890914.1, ZP_04891982.1, ZP_04893793.1, ZP_04902131.1, ZP_04905327.1, ZP_04941068.1, ZP_04944536.1, ZP_04945255.1, ZP_04959332.1, ZP_04964181.1, ZP_05053721.1, ZP_05063588.1, ZP_05073059.1, ZP_05077806.1, ZP_05082750.1, ZP_05091128.1, ZP_05095488.1, ZP_05101701.1, ZP_05116783.1, ZP_05121836.1, ZP_05127756.1, ZP_05637806.1, ZP_05742087.1, ZP_05783548.1, ZP_05786246.1, ZP_05843149.1, ZP_05945960.1, ZP_06459045.1, ZP_06487195.1, ZP_06492453.1, ZP_06493162.1, ZP_06703644.1, ZP_06731146.1, ZP_06839371.1, ZP_07007312.1, ZP_07266194.1, ZP_07374050.1, ZP_07662787.1, ZP_07778196.1, ZP_07797983.1, ZP_08099459.1, ZP_08138203.1, ZP_08141719.1, ZP_08142973.1, ZP_08177102.1, ZP_08185821.1, ZP_08186468.1, ZP_08208888.1, ZP_08266590.1, ZP_08402041.1, ZP_08406891.1, ZP_08522175.1, ZP_08527488.1, ZP_08631252.1, and ZP_08636687.1.

In particular, the Enzyme E_1, may be an aminotransferase selected from the group consisting of NP_901695.1, ZP_03697266.1, AAD41041.1, YP_002796201.1, ZP_03697962.1, YP_001859758.1, YP_002229759.1, YP_001120907.1, YP_110490.1, ZP_04964181.1, YP_774932.1, YP_001766294.1, YP_001581170.1, YP_622254.1, ZP_03588560.1, YP_001809596.1, YP_370582.1, ZP_03572809.1, NP_248990.1, YP_001888405.1, ZP_04905327.1, YP_001061726.1, YP_001668026.1, ZP_01750280.1, ZP_07778196.1, EGH71404.1, NP_744329.1, YP_004147141.1, ADR61066.1, ZP_05783548.1, YP_004701637.1, YP_366340.1, YP_003264235.1, EGD03176.1, YP_001268866.1, ZP_01901558.1, ZP_05121836.1, YP_003692877.1, ZP_03517291.1, YP_002974935.1, YP_001668026.1, ADR61066.1, NP_744329.1, YP_001268866.1, YP_004701637.1, ZP_08142973.1, ADR62525.1, YP_610700.1, NP_747283.1, ADR62525.1, YP_001270391.1, YP_004704442.1, YP_610700.1, YP_001747156.1, ZP_08138203.1, ZP_07266194.1, EGH70821.1, YP_351134.1, EGH32343.1, EGH08331.1, EGH67339.1, YP_001668026.1, YP_004701637.1, YP_237931.1, ZP_03400081.1, ZP_05116783.1, ZP_01550953.1, ZP_07662787.1, YP_928515.1, YP_788372.1, YP_001021095.1, ZP_07797983.1, YP_003578319.1, YP_004305252.1, NP_248912.1, ZP_08636687.1, YP_003912421.1, YP_751687.1, ZP_08142973.1, YP_271307.1, ZP_05082750.1, YP_001417624.1, and YP_353455.1.

Even more in particular, the Enzyme E_1 may be transaminase CV2025 from *Chromobacterium violaceum* DSM30191. The Enzyme E_1 according to any aspect of the present invention may comprise at least 50% sequence identity relative to SEQ ID NO:2. More in particular, E_1 may comprise a polypeptide with sequence identity of at least 50, 60, 65, 70, 75, 80, 85, 90, 91, 94, 95, 98 or 100% to a polypeptide SEQ ID NO:2.

The enzymes, for example E_1 may comprise a polypeptide sequence wherein up to 60%, preferably up to 25%, particularly up to 15%, in particular up to 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1% of the amino acid residues are modified compared to the below reference sequences (accession numbers by deletion, insertion, substitution or a combination thereof and which still possess at least 50%, preferably 65%, particularly preferably 80%, in particular more than 90% of the activity of the protein with the corresponding, below reference sequence, wherein 100% activity of the reference protein is understood to mean the increasing of the activity of the cells used as a biocatalyst, i.e. the quantity of substance converted per unit time based on the cell quantity used (units per gram cell dry weight [U/g CDW]) in comparison to the activity of the biocatalyst in the absence of the reference protein. Modifications of amino acid residues of a given polypeptide sequence which lead to no significant modifications of the properties and function of the given polypeptide are known to those skilled in the art. Thus for example many amino acids can often be exchanged for one another without problems; examples of such suitable amino acid substitutions are: Ala by Ser; Arg by Lys; Asn by Gln or His; Asp by Glu; Cys by Ser; Gln by Asn; Glu by Asp; Gly by Pro; His by Asn or Gln; Ile by Leu or Val; Leu by Met or Val; Lys by Arg or Gln or Glu; Met by Leu or Ile; Phe by Met or Leu or Tyr; Ser by Thr; Thr by Ser; Trp by Tyr; Tyr by Trp or Phe; Val by Ile or Leu. It is also known that modifications, particularly at the N- or C-terminus of a polypeptide in the form of for example amino acid insertions or deletions, often exert no significant influence on the function of the polypeptide.

The expression of the enzymes and genes mentioned above and all mentioned below is determinable by means of 1- and 2-dimensional protein gel separation followed by optical identification of the protein concentration in the gel with appropriate evaluation software.

If the increasing of an enzyme activity is based exclusively on increasing the expression of the corresponding gene, then the quantification of the increasing of the enzyme activity can be simply determined by a comparison of the 1- or 2-dimensional protein separations between wild type and genetically modified cell. A common method for the preparation of the protein gels with bacteria and for identification of the proteins is the procedure described by Hermann et al. (Electrophoresis, 22: 1712-23 (2001). The protein concentration can also be analysed by Western blot hybridization with an antibody specific for the protein to be determined (Sambrook et al., Molecular Cloning: a laboratory manual, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. USA, 1989) followed by optical evaluation with appropriate software for concentration determination

(Lohaus and Meyer (1989) Biospektrum, 5: 32-39; Lottspeich (1999), Angewandte Chemie 111: 2630-2647). This method is also always an option when possible products of the reaction to be catalysed by the enzyme activity to be determined may be rapidly metabolized in the microorganism or else the activity in the wild type is itself too low for it to be possible adequately to determine the enzyme activity to be determined on the basis of the production formation.

According to another aspect of the present invention, there is provided a method of producing at least one omega-hydroxy carboxylic acid and/or ester thereof from at least one carboxylic acid and/or ester thereof as substrate, the method comprising

- contacting at least one genetically modified cell expressing a mutant AlkB enzyme with the substrate carboxylic acid and/or ester thereof,

wherein the substrate carboxylic acid and/or ester thereof is a C_6-C_{14} carboxylic acid and/or ester thereof; and

the mutant AlkB enzyme comprises at least one point mutation in the wild type sequence of AlkB, wherein the point mutation is at amino acid position selected from the group consisting of V129, L132 and T136 of the wild type AlkB enzyme.

In particular, the point mutation may be selected from the group consisting of V129T, T136A, V129A and L132V.

According to another aspect of the present invention, there is provided a method of producing at least one omega-hydroxy carboxylic acid and/or ester thereof from at least one carboxylic acid and/or ester thereof as substrate, the method comprising

- contacting at the cell according to any aspect of the present invention with the substrate carboxylic acid and/or ester thereof.

According to a further aspect of the present invention, there is provided a method of producing at least one omega-aminocarboxylic acid and/or ester thereof from at least one carboxylic acid and/or ester thereof as substrate, the method comprising

- contacting the cell according to any aspect of the present invention with the substrate carboxylic acid and/or ester thereof.

According to yet a further aspect of the present invention, there is provided a use of the cell according to any aspect of the present invention for catalysing the conversion of a carboxylic acid and/or ester thereof to a corresponding omega-hydroxy carboxylic acid and/or ester thereof and/or omega-aminocarboxylic acid and/or ester thereof.

The term “contacting”, as used herein, means bringing about direct contact between the substrate carboxylic acid and/or ester thereof with the cell according to any aspect of the present invention in an aqueous solution. For example, the cell and carboxylic acid and/or ester thereof may not be in different compartments separated by a barrier such as an inorganic membrane. If the carboxylic acid and/or ester thereof is soluble or made soluble and may be taken up by the cell or can diffuse across biological membranes, it may simply be added to the cell according to any aspect of the present invention in an aqueous solution. In case it is insufficiently soluble, it may be solved in a suitable organic solvent prior to addition to the aqueous solution. The person skilled in the art is able to prepare aqueous solutions of carboxylic acid and/or ester thereof having insufficient solubility by adding suitable organic and/or polar solvents. Such solvents may be provided in the form of an organic phase comprising liquid organic solvent. In one example, the organic solvent or phase may be considered liquid when liquid at 25 °C and standard atmospheric pressure. In another example, the compounds and catalysts may be contacted in vitro, i.e. in a more or less enriched or even purified state, or may be contacted in situ, i.e. they are made as part of the metabolism of the cell and subsequently react inside the cell.

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

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A-1D are graphs with HLAME and DDAME concentrations after 29 h of biotransformation. HLAME: omega-hydroxylauric acid methylester; DDAME: dodecanedioic acid monomethylester; OLAME: omega-oxolauric acid methylester; LAME: lauric acid methyl ester.

EXAMPLES

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

Example 1

Site-directed mutagenesis.

The amino acid positions V129, L132 and T136 of AlkB were thought to be involved in substrate recognition and substrate positioning. In order to exchange amino acids in the AlkB enzyme, the corresponding base triplets coding for the desired amino acid were introduced into synthetic oligonucleotides. With the wildtype AlkB gene sequence as a PCR template the mutant AlkB genes (coding for AlkB mutants V129T, V129A and T136A, respectively) were amplified via PCR by the use of the synthetic oligonucleotides. The PCR products were used as insert DNA for the *in vitro* construction of the expression plasmids. Nucleotide exchanges were introduced via site-directed mutagenesis according to methods known to a person skilled in the art and are shown in the right column of Table 1. These exchanges led to the corresponding AlkB variants shown in the left column of Table 1.

AlkB enzyme variant	Nucleotide exchange in AlkB coding sequence
Wildtype alkB	SEQ ID NO:3
V129T	G385A, T386C, G387C
V129A	T386C, G387C
T136A	A406G, A408T

Table 1. Nucleotide sequences that were changed to produce specific AlkB mutants

Example 2

In vitro construction of expression plasmids

As backbone vector for plasmid construction the plasmid pBT10_alkL [for sequence and construction see Example 1 of WO/2011/131420 (Seq ID No: 8 of WO/2011/131420)] was used. In pBT10_alkL the wildtype AlkB sequence was replaced using the PCR products containing the mutant AlkB genes by the use of the NEBuilder® HiFi DNA Assembly Cloning Kit from NEB (New England Biolabs, Inc.) according to the manufacturer's specifications.

Plasmid	AlkB variant	Transaminase	SEQ ID NO:
pBT10_alkL	Wildtype	-	5
pBT10_alkL_V129T	V129T	-	6
pBT10_alkL_T136A	T136A	-	7
pBT10_alkL_V129A	V129A	-	8
pJ294_alaDH_B.s._TA _C.v.(Ct)	-	Cv_2025	4

Table 2. Plasmids which were used for the construction of *E. coli* strains. For construction of Plasmid pJ294_alaDH_B.s._TA_C.v.(Ct) see example 1 of WO/2013/024114

Example 3

Construction of E. coli strains

E. coli W3110 ΔfadE (no b-oxidation) ΔbioH (no methyl ester hydrolysis) was used. The *E. coli* W3110 ΔbioH strain to knockout the expression of bioH was made using the method disclosed in Example 1 of EP2730655. This strain was then subsequently used to knock out the expression of fadE using the method disclosed in Example 1 of EP2607490. The resultant strain was called *E.coli* W3110 ΔfadEΔbioH. For the construction of *E. coli* strains expressing wildtype AlkB, AlkB mutant genes (AlkB variants), and/or transaminase, the *E. coli* strain W3110 ΔfadEΔbioH was used as host for transformations via electroporation according to methods known to a person skilled in the art. Transformants were selected on LB agar plates containing Kanamycin (50 µg/ml) and/or ampicillin (100 µg/ml). The list of transformants is provided in Table 3.

***E. coli* strain**	**Plasmid 1**	**Plasmid 2**
EcW3110ΔfadEΔbioH_pBT10_alkL	pBT10_alkL	-
EcW3110ΔfadEΔbioH_pBT10_alkL_V129T	pBT10_alkL_V129T	-
EcW3110ΔfadEΔbioH_pBT10_alkL_T136A	pBT10_alkL_T136A	-
EcW3110ΔfadEΔbioH_pBT10_alkL_V129A	pBT10_alkL_V129A	-
EcW3110ΔfadEΔbioH_pBT10_alkL_TA	pBT10_alkL	pJ294_alaDH_B.s._TA_C.v.(Ct)
EcW3110ΔfadEΔbioH_pBT10_alkL_V129T_TA	pBT10_alkL_V129T	pJ294_alaDH_B.s._TA_C.v.(Ct)
EcW3110ΔfadEΔbioH_pBT10_alkL_T136A_TA	pBT10_alkL_T136A	pJ294_alaDH_B.s._TA_C.v.(Ct)
EcW3110ΔfadEΔbioH_pBT10_alkL_V129A_TA	pBT10_alkL_V129A	pJ294_alaDH_B.s._TA_C.v.(Ct)

Table 3. *E. coli* strains obtained by transformation of *E. coli* W3110 ΔfadEΔbioH with plasmid 1. *E. coli* strains with both plasmids (plasmid 1 and plasmid 2) are generated via transformation of both plasmids.

Example 4

Activity of AlkB mutants

The strains of Table 3 were subjected to a fed-batch fermentation in order to investigate their ability to produce omega-hydroxylauric acid methylester, omega-oxolauric acid methylester and dodecanedioic acid monomethylester from methyl laurate (lauric acid methylester). This was carried out in an 8-fold parallel fermentation system from DASGIP ®.

For the fermentation, 1 l reactors were used which were equipped with overhead stirrers and impeller turbines. To monitor the process, pH and pO2 were measured directly and continuously during the process using methods known in the art. OTR/CTR measurements served inter alia for estimating the metabolic activity and fitness of the cells.

The pH probes were calibrated by means of a two-point calibration with measurement solutions of pH 4.0 and pH 7.0 according to technical reference of DASGIP. The reactors were provided according to technical reference with the required sensors and connections and the stirrer shaft was installed. The reactors were then filled with 300 ml of water and autoclaved for 20 min at 121°C in order to ensure sterility. The pO2 probes were polarized overnight (at least 6 h) following connection to the measurement amplifier. The water was then removed under the clean bench and replaced by high-cell-density medium consisting of (NH4)2SO4 1.76 g/l, K2HPO4 19.08 g/l, KH2PO4 12.5 g/l, yeast extracts 6.66 g/l, trisodium citrate dihydrate 11.2 g/l, 17 ml/l of a filter-sterilized 1% strength ammonium iron citrate solution, and 5 ml/l of a filter-sterilized trace element stock solution (consisting of HCl (37%) 36.50 g/l, MnCl2*4H2O 1.91 g/l, ZnSO4*7H2O 1.87 g/l, ethylenediaminetetraacetic acid dihydrate 0.84 g/l, H3BO3 0.30 g/l, Na2MoO4*2H2O 0.25 g/l, CaCl2*2H2O 4.70 g/l, FeSO4*7H2O 17.80 g/l, CuCl2*2H2O 0.15 g/l) with 15 g/l glucose as carbon source (added by metered addition of 30 ml/l of a sterile feed solution consisting of 500 g/l glucose, 1% (w/v) MgSO4*7H2O and 2.2% (w/v) NH4Cl) with 50 mg/l kanamycin.

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

All of the aforementioned *E. coli* strains were cultured firstly from a cryoculture in LB medium (25 ml in a 100 ml baffled shake flask) with 50 mg/l kanamycin overnight at 37°C and 200 rpm for about 18 h. Then, 2 ml of this culture were transferred for a second preculture stage into 25 ml of high-cell-density medium consisting of (NH4)2SO4 1.76 g/L, K2HPO4 19.08 g/l, KH2PO4 12.5 g/l, yeast extract 6.66 g/l, trisodium citrate dihydrate 11.2 g/l, 17 ml/l of a filter-sterilized 1% strength ammonium iron citrate solution, and 5 ml/l of a filter-sterilized trace element stock solution (consisting of HCl (37%) 36.50 g/l, MnCl2*4H2O 1.91 g/l, ZnSO4*7H2O 1.87 g/l, ethylenediaminetetraacetic acid dihydrate 0.84 g/l, H3BO3 0.30 g/l. Na2MoO4*2H2O 0.25 g/l, CaCl2*2H2O 4.70 g/l, FeSO4*7H2O 17.80 g/l, CuCl2*2H2O 0.15 g/l) with 15 g/l glucose as carbon source (added by metered addition of 30 ml/l of a sterile feed solution consisting of 500 g/l glucose, 1% (w/v) MgSO4*7H2O and 2.2% (w/v) NH4Cl) with the already described antibiotics in a 100 ml shake flask and incubated at 37°C/200 rpm for a further 6 h.

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

The standard program used is shown in Table 4:

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

N (Rotation)	from	to	XO2 (gas mixture)	from	to	F (gas flow rate)	from	to
growth and biotransformation	0%	30%	growth and biotransformation	0%	100%	growth and biotransformation	15%	80%
	400 rpm	1500 rpm		21%	21%		6 sL/h	72 sL/h

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

Table 4. The standard program for determining activity of AlkB mutants

The pH was regulated to pH 6.8 on one side with 12.5% strength ammonia solution. During cultivation and biotransformation, the dissolved oxygen (pO2 or DO) in the culture was regulated to at least 30% by means of stirrer feed and gassing rate. Following inoculation, the DO dropped from 100% to this 30%, where it was kept stable for the remainder of the fermentation.

The fermentation was carried out as fed-batch, where the feed start was triggered as delivery to the feed phase with 5 g/l*h glucose feed, consisting of 500 g/l glucose, 1% (w/v) MgSO4*7H2O and 2.2% (w/v) NH4Cl, via the DO peak inducing the end of the batch phase. With feed start, the temperature of 37°C was lowered to 30°C. 10 h after feed start, the expression of the oxidation genes was induced with 0.025% (v/v) DCPK. The start of the methyl hydroxylaurate production (= start of the biotransformation) was carried out 14 h after feed start. For this purpose, 150 ml of a mixture of methyl laurate and oleic acid (technical-grade 90%) were added as batch to the fermentation broth. The results are shown in Figures 1A-1D. All mutants show faster HLAME production compared to control (2-3fold), faster overall oxidation compared to control (1.5-2fold) and an improved chemo selectivity (HLAME/DDAME ratio) in all mutants compared to control (1.20 – 1.75 vs. 0.84). OLAME production is low in all strains (1 g/L in V129A mutant, in all other strains < 0.6 g/L).

Example 5

LC-ESI/MS^2-based quantification of products

To quantify LAME and HLA in fermentation samples, samples were taken 1/2/4/20/22 h after the start of biotransformation. These samples were prepared for analysis. The quantification of educts, intermediates and products in fermentation samples was carried out by means of LC-ESI/MS^2 by reference to an external calibration for all analytes (0.1 – 50 mg/l) and using the internal standard aminoundecanoic acid (AUD for HLAME), and d3-LAME (for LAME).

The following instruments were used:

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

The samples were prepared by pipetting 1900 µl of solvent (80% (v/v) acetonitrile, 20% double-distilled H2O (v/v), + 0.1% formic acid) and 100 µl sample in a 2-ml reaction vessel. The mixture was vortexed for about 10 seconds and then centrifuged at about 13 000 rpm for 5 min. The clear supernatant was removed using a pipette and, after appropriate dilution, analysed with diluents (80% (v/v) ACN, 20% double-distilled. H2O (v/v), + 0.1% formic acid). 100 µL of ISTD were pipetted into each 900 µL sample (10 µL for a sample volume of 90 µL).

The HPLC separation was carried out with the aforementioned column and pre-column. The injection volume was 0.7 µL, the column temperature 50°C, the flow rate 0.6 mL/min. The mobile phase consisted of Eluent A (0.1% strength (v/v) aqueous formic acid) and Eluent B (acetonitrile with 0.1% (v/v) formic acid). The gradient profile as shown in Table 5 was used:

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

Table 5. Gradient profile used in HPLC separation

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

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

The detection and quantification of the compounds ALAME, DDAME, HLAME, OLAME, LAME, Di-OLAME was carried out with the following MRM parameters, with in each case a product ion being used as qualifier and one as quantifier.

Example 6

Activity of AlkB mutants with transaminase

The strains are subjected to a fed-batch fermentation as described in Example 5 in order to investigate their ability to produce omega-hydroxylauric acid methylester, omega-oxolauric acid methylester and dodecanedioic acid monomethylester from methyl laurate (lauric acid methylester). This is carried out in an 8-fold parallel fermentation system from DASGIP as described in example 4.

Example 7

LC-ESI/MS^2-based quantification of products of AlkB mutants with transaminase

The procedure for quantification of educts, intermediates and products is carried out as described in Example 5. Strains EcW3110ΔfadEΔbioH_pBT10_alkL_V129T_TA, EcW3110ΔfadEΔbioH_pBT10_alkL_T136A _TA and EcW3110ΔfadEΔbioH_pBT10_alkL_V129A_TA produce more ALAME relative to DDAME than the reference strain EcW3110ΔfadEΔbioH_pBT10_alkL_TA.

SEQUENCE LISTING

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gcaacaaatc cgtccctttt taaactctac aggccacgga ttacgtggcc tgtagacgtc 11940

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cccgttttac cagtccccta tcgccgcctg agagggcgca ggaagcgagt aatcagggta 12120

tcgaggcgga ttcacccttg gcgtccaacc agcggcacca gcggcgcctg agaggcgaat 12180

tgacataagc ctgttcggtt cgtaaactgt aatgcaagta gcgtatgcgc tcacgcaact 12240

ggtccagaac cttgaccgaa cgcagcggtg gtaacggcgc agtggcggtt ttcatggctt 12300

gttatgactg ttttttgta cagtctatgc ctcgggcatc caatcgat 12348

CLAIMS

1. A microbial cell expressing a mutant AlkB enzyme, the mutant AlkB enzyme comprising at least one point mutation in the wild type sequence of AlkB, wherein the point mutation is at amino acid position V129 and/or T136 of the wild type AlkB enzyme,
wherein the point mutation at amino acid V129 is selected from the group consisting of V129T and V129A,
wherein the point mutation at amino acid position T136 is T136A, and
wherein the wild type AlkB comprises a polypeptide with sequence identity of at least 80% to a polypeptide SEQ ID NO: 1.

2. The cell according to claim 1, wherein the point mutation is at amino acid position T136.

3. The cell according to either claim 1, wherein the wild type AlkB comprises a polypeptide with sequence identity of at least 90% to a polypeptide SEQ ID NO: 1.

4. The cell according to claim 1, wherein the wild type AlkB comprises a polypeptide with sequence identity of at least 95% to a polypeptide SEQ ID NO: 1.

5. The cell according to claim 1, wherein the point mutation is at amino acid position V129.

6. The cell according to claim 1, wherein the point mutation is at amino acid positions V129 and T136.

7. The cell according to claim 1, wherein the wild type AlkB comprises a polypeptide with sequence identity of at least 98% to a polypeptide SEQ ID NO: 1.

8. The cell according to any one of the preceding claims, wherein the wild type sequence of AlkB is SEQ ID NO:1.

9. The cell according to any one of the preceding claims, wherein the cell is further genetically modified to comprise increased expression relative to a wild type cell of
   - an enzyme E_1, an ω-transaminase capable of catalysing the conversion of the omega-oxocarboxylic acids and/or esters thereof to the corresponding omega-aminocarboxylic acids and/or esters thereof.

10. A method of producing at least one omega-hydroxy carboxylic acid and/or ester thereof from at least one carboxylic acid and/or ester thereof as substrate, the method comprising
   - contacting at least one genetically modified cell expressing a mutant AlkB enzyme with the substrate carboxylic acid and/or ester thereof,

wherein the substrate carboxylic acid and/or ester thereof is a C_6-C_{14} carboxylic acid and/or ester thereof; and

the mutant AlkB enzyme comprises at least one point mutation in the wild type sequence of AlkB,

wherein the point mutation is selected from the group consisting of V129T, T136A, V129A and L132V.

wherein the wild type AlkB comprises a polypeptide with sequence identity of at least 80% to a polypeptide SEQ ID NO: 1.

11. The method according to claim 10, wherein the wild type AlkB comprises a polypeptide with sequence identity of at least 90% to a polypeptide SEQ ID NO: 1.

12. The method according to claim 10, wherein the wild type AlkB comprises a polypeptide with sequence identity of at least 95% to a polypeptide SEQ ID NO: 1.

13. The method according to claim 10, wherein the wild type AlkB comprises a polypeptide with sequence identity of at least 98% to a polypeptide SEQ ID NO: 1

14. The method according to any one of claims 10 to 13, wherein the wild type sequence of AlkB is SEQ ID NO:1.

15. The method according to any one of claims 10 to 14, wherein the substrate carboxylic acid and/or ester thereof is lauric acid and/or lauric acid methyl ester (LAME) and the corresponding omega-hydroxy carboxylic acid and/or ester thereof is omega-hydroxylauric acid and/or omega-hydroxylauric acid methyl ester (HLAME).

16. A method of producing at least one omega-hydroxy carboxylic acid and/or ester thereof from at least one carboxylic acid and/or ester thereof as substrate, the method comprising

- contacting at least one genetically modified cell according to any of the claims 1 to 7 with the substrate carboxylic acid and/or ester thereof.

17. A method of producing at least one omega-aminocarboxylic acid and/or ester thereof from at least one carboxylic acid and/or ester thereof as substrate, the method comprising

- contacting the cell according to claim 9 with the substrate carboxylic acid and/or ester thereof.

18. Use of the cell according to any one of claims 1 to 8, for catalysing the conversion of a carboxylic acid and/or ester thereof to a corresponding omega-hydroxy carboxylic acid.

19. Use of the cell according to claim 9, for catalysing the conversion of a carboxylic acid and/or ester thereof to a corresponding omega-amino carboxylic acid and/or ester thereof.