

Production of ω -Amino Fatty Acids

The invention relates to a whole cell catalyst which expresses a recombinant α -dioxygenase or
5 the combination of a recombinant fatty acid reductase and of a phosphopantetheinyl transferase
phosphopantetheinylating the fatty acid reductase, and which additionally expresses a
transaminase, characterized in that the phosphopantetheinyl transferase and/or transaminase is
preferably recombinant; and a method for the conversion of a fatty acid, ω -hydroxy fatty acid,
10 ω -oxo fatty acid or a monoester thereof to an amine, comprising the steps oxidation of the fatty
acid, ω -hydroxy fatty acid, ω -oxo fatty acid or the monoester thereof to an oxidation product by
contacting with an alkane hydroxylase and/or alcohol dehydrogenase, contacting the oxidation
product with a phosphopantetheinylated fatty acid reductase or a α -dioxygenase to give an
aldehyde, and contacting the aldehyde with a transaminase.

15 Polyamides are a class of polymer which are characterized by repeating amide groups. The
term "polyamide" in contrast to the chemically related proteins, usually relates to synthetic,
commercially available thermoplastic plastics. Polyamides are derived from primary amines or
from secondary amines, which are conventionally obtained in the cracking of hydrocarbons.
However, derivatives, more precisely aminocarboxylic acids, lactams and diamines, can also be
20 used for polymer production. Also of interest are short-chain, gaseous alkanes as educts which
can be obtained starting from renewable raw materials by biotechnological methods.

Many polyamides in great commercial demand are produced starting from lactams. For example
"polyamide 6" can be obtained by polymerization of ϵ -caprolactam and "polyamide 12" by
25 polymerization of laurilactam. Other commercially interesting products include copolymers of
lactams, e.g. copolymers of ϵ -caprolactam and laurilactam.

The conventional industrial chemical production of amines is dependent on the supply of fossil
raw materials, and inefficient, and in the process large quantities of undesired by-products are
30 formed, in many steps of the synthesis up to 80%. One example of such a process is the
production of laurilactam. Conventionally, this is effected via a multistage process which not
only gives a low yield, but at the same time necessitates the provision of a costly infrastructure.

In view of these disadvantages, methods were developed for obtaining amines with the use of
35 biocatalysts starting from renewable raw materials. Possible renewable raw materials are in

particular sources of fatty acids, which can be obtained in the form of rape oil, great globe thistle oil, palm nut oil, coconut oil, sunflower oil and similar natural products from a multitude of biological sources, in particular from plants.

- 5 PCT/EP2008/067447 describes a biotechnological system for the production of chemically related products, more precisely ω -aminocarboxylic acids, with the use of a cell which has a range of suitable enzymatic activities and is capable of converting carboxylic acids to corresponding ω -aminocarboxylic acids. The process comprises a cascade of enzymatically catalysed reactions, in particular the oxidation of a fatty acid at the terminal carbon atom to the
- 10 aldehyde, and subsequent amination with the use of a transaminase and an amino acid as amine donor, which can be regenerated via an amino acid dehydrogenase.

However, a known disadvantage of the AlkBGT oxidase system from *Pseudomonas putida* GPO1 used therein consists in that it is not capable of performing a selective oxidation of

15 aliphatic alkanes to primary alcohols. Rather, a large number of oxidation products are formed, in particular the proportion of more highly oxidised products such as the corresponding aldehyde, ketone or the corresponding carboxylic acid increases with increasing reaction time (C. Grant, J. M. Woodley and F. Baganz (2011), *Enzyme and Microbial Technology* 48, 480-486), which correspondingly reduces the yield of desired amine.

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The problem of the relatively unselective oxidation is worsened by the fact that the oxidation products which form are structurally very similar. This means that it is very difficult to separate them from the desired oxidation products efficiently and without significant loss of yield.

- 25 A further disadvantage of this method consists in that overoxidized by-products, for example the dicarboxylic acid of the fatty acid used as educt, the recycling of hydrophobic solvents and hydrophobic liquid cation exchangers, which according to PCT/EP2011/071491 are used for the separation of the product from the aqueous reaction mixture, are at the expense of the efficiency in resource utilization.

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In this connection, it should be emphasized that the complexity of biotechnological systems with a cascade of reactions as described in PCT/EP2008/067447, each case reaction of which is catalyzed by one specific enzyme, renders the optimization of the reaction conditions difficult. Thus in the case of the essentially reactive ω -amino fatty acids as product, there is the

possibility that beyond a certain critical concentration in the interior of the cell they will react with essential components of the organism and thus have a toxic effect. If that is the case, then the growth and synthetic ability of the organism is impaired even leading to the death of the cell, without the developer being able directly to recognize the toxicity or even to attribute it to a specific educt, intermediate or product. It is also not predictable which organism tolerates what concentration of a chemically reactive substance.

Also with reference to a product yield to be improved and formation of by-products to be decreased, those skilled in the art cannot routinely identify limiting and critical factors in a system such as that described in PCT/EP2008/067447. If the yield of product is too low, then this can be because one of the enzymes is present in too low a concentration, without it being known which of the possible enzymes this is, i.e. owing to insufficient synthetic capacity the educt is not converted in the predicted time periods or before degradation by competing enzymes. Alternatively, it is entirely possible that an enzyme is indeed detectable in the cell in the form of a polypeptide, but in this particular cell does not have the folding essential for the activity, or a cofactor hitherto unknown but essential for the activity is lacking. Likewise, as already mentioned, the metabolic product can be toxic to the cell, or be degraded. Finally, interfering interactions with endogenous enzymes, i.e. those naturally present in a cell used as a whole cell catalyst, must be reckoned with.

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There is thus a need for processes for the production of ω -amino fatty acids from fatty acids, wherein the enzymatically catalyzed reactions proceed more selectively and the formation of undesired by-products is minimized.

25 Against this background, the problem on which the invention is based is to provide as efficient as possible a biotechnological process for the production of ω -amino fatty acids with regard to yield, carbon and/or nitrogen balance and/or purity.

A further problem on which the invention is based consists in providing as efficient as possible a biotechnological process for the conversion of carboxylic acid esters to aminated carboxylic acid esters with regard to yield, carbon and/or nitrogen balance, reusability of agents used and/or purity of the product. In this context, an efficient carbon and/or nitrogen balance is preferably understood to mean that as high as possible a proportion of the carbon and/or nitrogen fed to a cell for the conversion of a carboxylic acid ester in the form of suitable substrates is recovered

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in the desired end product, instead of for example being converted to products other than that desired.

A further problem on which the invention is based consists in improving the processability of a multiphase reaction mixture from the conversion of a carboxylic acid ester, particularly with regard to the reusability of the hydrophobic solvents and liquid cation exchangers used for the processing, and with regard to the phase formation and separation in a biphasic system comprising an aqueous phase, in which the conversion of the carboxylic acid ester proceeds, and an organic phase with organic solvents and/or liquid cation exchangers.

These and other problems are solved by the subject matter of the present application and in particular by the subject matter of the appended independent claims, wherein embodiments follow from the subclaims.

Summary

In one aspect, the present disclosure refers to a whole cell catalyst which comprises a transaminase and one selected from the group consisting of

1) an α -dioxygenase and

2) a combination of 2a) a recombinant fatty acid reductase and 2b) a phosphopantetheinyl transferase,

wherein:

the phosphopantetheinyl transferase phospho-pantetheinylates the fatty acid reductase, thereby forming a phosphopantetheinylated fatty acid reductase,

the fatty acid reductase catalyzes the conversion of an ω -carboxy acid to a corresponding ω -oxo fatty acid,

at least one of the phosphopantetheinyl transferase and transaminase is recombinant, when the catalyst comprises the transaminase and the α -dioxygenase, the transaminase is overexpressed, and when the catalyst comprises the transaminase and the combination of

2a) a recombinant fatty acid reductase and 2b) a phosphopantetheinyl transferase, the transaminase and/or the phosphopantetheinyl transferase is overexpressed.

5 In one example, the problem on which the disclosure is based is solved by a whole cell catalyst which expresses a recombinant α -dioxygenase or the combination of a recombinant fatty acid reductase and of a phosphopantetheinyl transferase phosphopantetheinylating the fatty acid reductase, and which additionally expresses a transaminase, wherein the phosphopantetheinyl transferase and/or transaminase is preferably recombinant.

10 In another example, the problem is solved by a whole cell catalyst which additionally expresses an amino acid dehydrogenase which is preferably recombinant.

In another example, the problem is solved by a whole cell catalyst which additionally expresses an alkane hydroxylase which is preferably recombinant.

15 In another example, the problem is solved by a whole cell catalyst which additionally expresses a polypeptide of the AlkL family which is preferably recombinant.

In another example, the problem is solved by a whole cell catalyst which additionally expresses an alcohol dehydrogenase which is preferably recombinant.

In another example, the problem is solved by a whole cell catalyst wherein the activity of at least one enzyme involved in β -oxidation is decreased compared to the wild type of the whole cell catalyst.

In another example, the problem is solved by a whole cell catalyst wherein the activity of BioH or a variant thereof is decreased compared to the wild type of the whole cell catalyst.

In another example, the problem is solved by a whole cell catalyst wherein the activity of FadL or a variant thereof is increased compared to the wild type of the whole cell catalyst.

In another aspect, the present disclosure refers to a method for preparing an amine, the method comprising:

- a) oxidizing a starting material comprising a fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or monoester thereof by contacting the starting material with an alkane hydroxylase, an alcohol dehydrogenase, or a mixture thereof to obtain an oxidation product;
- b) contacting the oxidation product with a phosphopantetheinylated fatty acid reductase or an α -dioxygenase to obtain an aldehyde; and
- c) contacting the aldehyde with a transaminase to obtain the amine,

wherein the phosphopantetheinylated fatty acid reductase or the α -dioxygenase in addition to the transaminase is provided in the form of the whole cell catalyst as disclosed herein.

In another example, the problem on which the disclosure is based is solved by a process for the conversion of a fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or a monoester thereof to an amine, comprising the steps

- 5 a) oxidation of the fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or the monoester thereof to an oxidation product by contacting with an alkane hydroxylase and/or alcohol dehydrogenase,
- 10 b) contacting the oxidation product with a phosphopantetheinylated fatty acid reductase or an α -dioxygenase to give an aldehyde, and
- c) contacting the aldehyde with a transaminase.

15 In another example, the problem on which the disclosure is based is solved by a process wherein in step c) an amino acid dehydrogenase is present.

In another example, the problem is solved by a process wherein at least one enzyme from the group comprising phosphopantetheinylated fatty acid reductase,

α -dioxygenase, transaminase, amino acid dehydrogenase and alkane hydroxylase, preferably all enzymes used from this group, are provided in the form of a whole cell catalyst according to an example of the disclosure.

- 5 In another example, the problem is solved by a process wherein the fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or the monoester thereof is a compound of the formula (I)



- 10 wherein R^1 is selected from the group which comprises -H, -CHO, -OH and $COOR^3$,

wherein R^2 and R^3 each and independently of one another are selected from the group which comprises H, methyl, ethyl and propyl,

- 15 with the proviso that at least one of the residues R^2 and R^3 is H, and

wherein A represents an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group with at least four carbon atoms.

- 20 In another example, the problem is solved by a process wherein A has the formula $-(CH_2)_n-$, wherein n is at least 4, preferably at least 10.

In another aspect, the present disclosure refers to a method for preparing an amine, the method comprising:

- 25
- a) oxidizing a starting material comprising a fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or monoester thereof by contacting the starting material with an alkane hydroxylase, an alcohol dehydrogenase, or a mixture thereof to obtain an oxidation product;
 - 30 b) contacting the oxidation product with a phosphopantetheinylated fatty acid reductase or an α -dioxygenase to obtain an aldehyde; and

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c) contacting the aldehyde with a transaminase to obtain the amine;

wherein at least one enzyme of a), b) and c) is present in the form of the whole cell catalyst as disclosed herein.

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In another example, the problem on which the disclosure is based is solved by use of the whole cell catalyst according to an example or of the process according to an example for the amination of a fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or a monoester thereof.

10 In another example, the problem on which the disclosure is based is solved by a reaction mixture comprising the whole cell catalyst according to an example in aqueous solution and a fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or a monoester thereof of the formula (I)



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wherein R^1 is selected from the group which comprises -H, -CHO, -OH and COOR^3 ,

wherein R^2 and R^3 each and independently of one another are selected from the group which comprises H, methyl, ethyl and propyl,

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with the proviso that at least one of the residues R^2 and R^3 is H, and

wherein A represents an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group with at least four carbon atoms, preferably the formula - $(\text{CH}_2)_n$ -,
 wherein n is at least 4, particularly preferably at least 10.

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In another aspect, the present disclosure refers to a reaction mixture comprising:

water;

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the whole cell catalyst as disclosed herein; and

a fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or a monoester thereof of the formula (I)

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wherein

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R^1 is -H, -CHO, -OH and COOR^3 ,

R^2 and R^3 are each independently H, methyl, ethyl or propyl, with the proviso that at least one of the residues R^2 and R^3 is H, and

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A is an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group with at least four carbon atoms.

The present disclosure is based on the discovery of the inventors that a functionally coexpressed recombinant fatty acid reductase or α -dioxygenase in a whole cell catalyst which is

used for the production of ω -amino fatty acids from fatty acids and has an appropriate enzyme composition, surprisingly increases the yield of ω -amino fatty acids.

Furthermore, the present disclosure is based on the discovery of the inventors that a functionally coexpressed recombinant fatty acid reductase or α -dioxygenase in a whole cell catalyst which is used for the production of ω -amino fatty acids from fatty acids and has an appropriate enzyme composition, surprisingly decreases the concentration of interfering by-products, in particular of overoxidized fatty acids in the form of dicarboxylic acids and esters thereof, in the product obtained.

Furthermore, the present disclosure is based on the discovery of the inventors that a functionally coexpressed recombinant fatty acid reductase or α -dioxygenase in a whole cell catalyst which is used for the production of ω -amino fatty acids from fatty acids and has an appropriate enzyme composition, improves the purity and reusability of liquid cation exchangers such as oleic acid, which are used for the removal of an ω -amino fatty acid from a fermentation solution containing the whole cell catalyst.

The present invention relates to an improved process for the conversion of a fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or a monoester thereof to an amine, which is distinguished

in that as well as the enzymes which catalyse the conversion of the fatty acid via its various oxidation stages to the amine, at least one fatty acid reductase or α -dioxygenase, or the combination of both enzymes, is also present, preferably, when a whole cell catalyst is used for performing the process. In a preferred embodiment, the term "fatty acid reductase", as used

5 herein, is understood to mean, an enzyme which catalyses the conversion of an ω -carboxy acid, also described as dicarboxylic acid or ω -carboxy fatty acid, to the corresponding ω -oxo fatty acid with consumption of ATP and NAD(P)H. In the state of the art, for example in WO/2010/135624, fatty acid reductases for the production of ω -hydroxy fatty acids are described, but not as part of a system for production of ω -amino fatty acids. In a still more

10 preferred embodiment, the fatty acid reductase is selected from the group of fatty acid reductases which contain the amino acid sequences YP_887275.1, ZP_11001941.1, ZP_06852401.1, NP_959974.1, YP_001070587.1, ZP_05217435.1, YP_882653.1, YP_639435.1, ZP_10800193.1, YP_006452763.1, YP_006730440.1, ZP_11196216.1, YP_005349252.1, ZP_05224908.1, YP_005338837.1, YP_006307000.1, YP_005343991.1,

15 ZP_11001942.1, ZP_09979565.1, YP_005003162.1, YP_953393.1, YP_001850422.1, ZP_11011489.1, ZP_12689264.1, YP_905678.1, ZP_09976919.1, YP_004746059.1, NP_217106.1, YP_004525443.1, NP_337166.1, ZP_09685823.1, YP_978699.1, ZP_06437984.1, ZP_06514086.1, NP_856267.1, CAA19077.1, NP_301424.1, ZP_06522140.1, ZP_06518098.1, ZP_11008938.1, ZP_07432374.2, AAR91681.1, YP_006808747.1,

20 YP_001851230.1, ZP_15327751.1, ZP_15455857.1, ZP_12874284.1, ZP_15332534.1, ZP_15512956.1, ZP_14244106.1, ZP_15470899.1, ZP_11439367.1, YP_001703694.1, ZP_15446742.1, YP_006808978.1, ZP_07964926.1, YP_006521379.1, WP_007769435.1, ZP_15512957.1, ZP_12874283.1, YP_005350955.1, ZP_14243341.1, YP_001705436.1, ZP_15329649.1, YP_006522325.1, YP_006732197.1, YP_003658971.1, ZP_05227804.1,

25 YP_001703695.1, YP_006308707.1, ZP_15342047.1, YP_006521380.1, ZP_15327752.1, YP_005340557.1, ZP_11439578.1, ZP_15392943.1, ZP_15514789.1, ZP_12996178.1, ZP_09412214.1, ZP_06849686.1, YP_889972.1, YP_006570321.1, ZP_15375693.1, YP_006308219.1, YP_006521600.1, YP_005340029.1, YP_005350457.1, ZP_11439836.1, ZP_12994664.1, ZP_14240588.1, ZP_14236860.1, ZP_09410830.1, YP_006731697.1,

30 YP_005264225.1, YP_001704097.1, ZP_15328186.1, ZP_09402885.1, ZP_12690463.1, AFO59871.1, ZP_07966879.1, YP_118225.1, YP_001828302.1, YP_006566873.1, YP_003660169.1, ZP_15337407.1, ZP_08240521.1, ZP_10456477.1, YP_001537947.1, YP_004016539.1, ZP_07664024.1, ZP_14244107.1, ZP_09794557.1, ZP_09274211.1, ZP_05224899.1, ZP_15484175.1, AAA17105.1, ZP_11437924.1, ZP_15446621.1,

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 10 YP_005348983.1, ZP_15472813.1, ZP_15457007.1, ZP_15421152.1, ZP_15488933.1,
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 ZP_12876400.1, ZP_12995435.1, ZP_07667680.1, YP_001281387.1, EIE21044.1,
 ZP_15375054.1, NP_334518.1, 4DQV_A, ZP_06435375.1, YP_003030020.1, YP_976237.1,
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 YP_884815.1, YP_884815.1,
 CAB55600.1, ZP_09081423.1, YP_006521568.1, ZP_11440626.1, ZP_15513309.1,
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 20 ZP_12873916.1, ZP_14242094.1, ZP_12994610.1, ZP_07664023.1, ZP_15446620.1,
 ZP_15484174.1, ZP_14240245.1, YP_005358845.1 and XP_002669159.1,
 in particular YP_006731697.1, ZP_09839660.1, YP_001704097.1, YP_889972.1,
 ZP_05045132.1, NP_959974.1, ZP_10456477.1, YP_118225.1, YP_905678.1, YP_887275.1,
 ZP_11001941.1, WP_007769435.1 and YP_005349252.1
 25 and variants thereof.

Fatty acid reductases are a group of enzymes which for their activity require a
 phosphopantetheinylation, i.e. the covalent binding of a phosphopantetheinyl cofactor on the
 enzymes. Accordingly, the fatty acid reductase used according to the invention is
 30 phosphopantetheinylated, and a whole cell catalyst expressing the fatty acid reductase
 expresses, either as part of its composition of endogenously expressed enzymes or in
 recombinant form, a phosphopantetheinyl transferase phosphopantetheinylating the fatty acid
 reductase. In a preferred embodiment, the term "phosphopantetheinyl transferase", as used
 herein, is understood to mean an enzyme which transfers a phosphopantetheinyl residue from a

phosphopantetheinyl-CoA to an enzyme, preferably onto the fatty acid reductase. In a particularly preferred embodiment, the phosphopantetheinyl transferase is selected from the group of phosphopantetheinyl transferases which contain the amino acid sequences

ABI83656.1, YP_006811024.1, YP_120266.1, YP_005265173.1, YP_004006671.1,
5 ZP_08152482.1, ZP_11104141.1, ZP_14482198.1, YP_706581.1, ZP_10002626.1,
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10 ZP_06852853.1, YP_953148.1, ZP_11011170.1, YP_639258.1, YP_886985.1,
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20 ZP_11236665.1, NP_630748.1, ZP_06527138.1, YP_003835167.1, CCH33620.1,
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25 YP_004015869.1, ZP_08801530.1, ZP_10550999.1, YP_004492879.1, ZP_09958730.1,
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30 ZP_07307765.1, ZP_07315112.1, YP_005466392.1, NP_824081.1, YP_003493882.1,
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 5 YP_004605750.1, ZP_03918977.1, AAF71762.1, ZP_05007864.1, ZP_06836265.1,
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 10 YP_001800249.1, YP_001157632.1, YP_166099.1, ZP_10088015.1, YP_004760065.1,
 ZP_07947675.1, YP_001603066.1, YP_003812683.1, YP_004403402.1, ZP_08292153.1,
 ZP_09471260.1, YP_004018108.1, ZP_05115352.1, AAD13565.1, ZP_09295321.1,
 YP_001535629.1, ZP_04607273.1, YP_006561753.1, ZP_00960958.1, YP_006571985.1,
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 15 YP_614397.1, ZP_11163860.1, YP_003983492.1, YP_004080668.1, ZP_09420475.1,
 ZP_05914565.1, ZP_01101149.1, ZP_14743088.1, YP_001239694.1, ZP_09127532.1,
 YP_003833873.1, ZP_08516197.1, ZP_10160483.1, ZP_01987188.1, ZP_01755304.1,
 ZP_08825027.1, ZP_05077116.1, YP_001444606.1, ZP_03392800.1, ZP_01057781.1,
 AFB69889.1, ZP_08815097.1 and AAO17175.1 and variants thereof. In a particularly preferred
 20 embodiment here, it is the phosphopantetheinyl transferase with the database code ABI83656.1
 or a variant thereof.

Alternatively or in addition to the combination of fatty acid reductase and phosphopantetheinyl
 transferase, the whole cell catalyst can also contain an α -dioxxygenase. In a preferred
 25 embodiment, the term " α -dioxxygenase", as used herein, is understood to mean an enzyme
 which catalyses the conversion of a fatty acid with the consumption of one molecule of oxygen
 and with cleavage of one carbon dioxide molecule to a fatty acid shortened by one carbon atom
 at the terminal ω carbon atom compared to the fatty acid used as educt, and bearing an
 aldehyde group on the terminal ω carbon atom. In a particularly preferred embodiment, the
 30 α -dioxxygenase is selected from the group of α -dioxxygenases which contain the amino acid
 sequences NP_001066718.1, EAY82977.1, BAH79993.1, ABG22011.1, BAJ90503.1,
 AFD04418.1, AFD04417.1, BAJ87736.1, AFW75180.1, ABG22012.1, XP_002311389.1,
 CAH05011.1, XP_002279884.1, CBI34957.3, AAG59584.1, NP_001234414.1,
 NP_001234410.1, XP_003553942.1, XP_002275161.1, XP_003553937.1, CBI34960.3,

- CAA07589.1, XP_003543402.1, XP_002517402.1, XP_002882184.1, NP_186791.1, AAK85133.1, CAN77070.1, XP_002529555.1, CAH64542.1, NP_001234061.1, XP_002281357.1, ADM21465.1, XP_002318527.1, NP_177509.1, CAN74266.1, XP_002888940.1, NP_001185393.1, XP_003631072.1, BAJ33800.1, XP_002517377.1, 5 XP_003530944.1, BAJ34623.1, ABG22013.1, ABP02610.1, XP_001773135.1, XP_002960339.1, ABK95279.1, ABD73303.1, ABD73304.1, YP_001805721.1, ZP_08971815.1, ZP_08430366.1, YP_823013.1, ZP_05026427.1, ZP_11003953.1, YP_007064484.1, YP_007113008.1, YP_633369.1, ZP_18906570.1, ZP_09251410.1, ZP_10050808.1, ZP_01306662.1, YP_001516886.1, ZP_05042862.1, AAC49625.1, 10 ZP_09648375.1, ZP_09792714.1, ZP_09788527.1, XP_001728273.1, AAC83355.1, YP_890542.1, ZP_11000891.1, XP_002605323.1, EGO58341.1, YP_006249145.1, YP_001507004.1, YP_001704637.1, ZP_12876141.1, ZP_11150830.1, ZP_14236257.1, ZP_09411385.1, ZP_14243118.1, EKD16664.1, ZP_15416799.1, ZP_15338016.1, ZP_10080295.1, ZP_11438929.1, ZP_12995210.1, ZP_10946648.1, YP_003409541.1, 15 XP_001637870.1, YP_005451221.1, XP_001212758.1, ZP_07290489.1, ZP_05781329.1, ZP_19187748.1, ZP_06574534.1, XP_002605322.1, NP_822950.1, YP_006366425.1, EJP63377.1, EKD21217.1, XP_001795927.1, XP_003042615.1, ZP_06566152.1, EGU88116.1, EFY94417.1, XP_388327.1, EKJ68934.1, ZP_07290463.1, CCC10458.1, YP_001107201.1, XP_003348248.1, T49753, CAD31840.1, XP_001229975.1, CBN77040.1, 20 YP_004813753.1, XP_002513273.1, XP_001627136.1, AFG52858.1, AFG52857.1, AEW08450.1, NP_841291.1, YP_004512343.1, ACG75701.1 and ZP_03500906.1 and variants thereof. In a particularly preferred embodiment, it is the α -dioxygenase with the database code NP_001066718.1 or a variant thereof.
- 25 As well as the α -dioxygenase or the combination of fatty acid reductase and the phosphopantetheinyl transferase, the whole cell catalyst according to the invention necessarily contains a transaminase which aminates the ω -oxo fatty acid. In a preferred embodiment, the term "transaminase", as used herein, is understood to mean an enzyme which catalyses the transfer of α amino groups from a donor molecule, preferably an amino acid, to an acceptor molecule, 30 preferably an α -keto carboxylic acid. In a particularly preferred embodiment, the transaminase is selected from the group of transaminases which contain the amino acid sequences 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,
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 10 YP_001765463.1, YP_001766294.1, YP_001790770.1, YP_001808775.1, YP_001809596.1,
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 15 YP_003591946.1, YP_003607814.1, YP_003641922.1, YP_003674025.1, YP_003692877.1,
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 20 YP_004701580.1, YP_004701637.1, YP_004704442.1, YP_108931.1, YP_110490.1,
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 25 YP_625753.1, YP_680590.1, YP_751687.1, YP_767071.1, YP_774090.1, YP_774932.1,
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 ZP_08208888.1, ZP_08266590.1, ZP_08402041.1, ZP_08406891.1, ZP_08522175.1,
 ZP_08527488.1, ZP_08631252.1, ZP_08636687 and variants thereof.

15 The fatty acid reductase used according to the invention and preferably also other enzymes
 used according to the invention are recombinant enzymes. In a preferred embodiment, the term
 "recombinant", as used herein, is understood to mean that the nucleic acid molecule coding for
 the corresponding enzyme does not occur in the natural cell and/or it was produced with use of
 genetic engineering methods. In a preferred embodiment, the term recombinant protein is used
 20 when the corresponding polypeptide is encoded by a recombinant nucleic acid. In a preferred
 embodiment, a recombinant cell, as used herein, is understood to be a cell which contains at
 least one recombinant nucleic acid or one recombinant polypeptide. Suitable methods for the
 production of recombinant molecules or cells are known to those skilled in the art, for example
 those described in Sambrook/Fritsch/Maniatis (1989): Molecular Cloning: A Laboratory Manual,
 25 Cold Spring Harbor Laboratory Press, 2nd edition. Recombinant enzymes are preferably
 overexpressed, for example with the use of pET or pGEX vector systems, which are known to
 those skilled in the art.

30 With regard to the choice of the organism, the whole cell catalyst usable according to the
 invention is subject to no restrictions, provided that it is culturable, stable and accessible if
 necessary to modifications introducible by genetic engineering, e.g. methods for the attenuation
 of enzyme activities, for example knockouts. Thus it can equally be a prokaryotic or a eukaryotic
 cell. In the case of a eukaryotic cell, unicellular eukaryotes are particularly preferable,
 particularly yeasts such as *Saccharomyces cerevisiae*, *Candida tropicalis*, *Candida albicans*

and *Pichia pastoris*. In the case of prokaryotic cells it can for example be a bacterium which is selected from the group which comprises *Magnetococcus*, *Mariprofundus*, *Acetobacter*, *Acetobacterium*, *Acidiphilium*, *Afipia*, *Ahrensia*, *Asticcacaulis*, *Aurantimonas*, *Azorhizobium*, *Azospirillum*, *Bacillus*, *Bartonella tribocorum*, *Beijerinckia*, *Bradyrhizobium*, *Brevundimonas*,
5 *subvibrioides*, *Brucella*, *Caulobacter*, *Chelativorans*, *Citricella*, *Citromicrobium*, *Clostridium*, *Corynebacterium*, *Dinoroseobacter*, *Erythrobacter*, *Fulvimarina*, *Gluconacetobacter*, *Granulibacter*, *Hirschia*, *Hoeflea*, *Hyphomicrobium*, *Hyphomonas*, *Ketogulonicigenium*, *Labrenzia*, *Loktanelia*, *Magnetospirillum*, *Maricaulis*, *Maritimibacter*, *Mesorhizobium*, *Methylobacterium*, *Methylocystis*, *Methylosinus*, *Nitrobacter*, *Novosphingobium*, *Oceanibulbus*,
10 *Oceanicaulis*, *Oceanicola*, *Ochrobactrum*, *Octadecabacter*, *Oligotropha*, *Paracoccus*, *Parvibaculum*, *Parvularcula*, *Pelagibaca*, *Phaeobacter*, *Phenylobacterium*, *Polymorphum*, *Pseudovibrio*, *Rhodobacter*, *Rhodomicrobium*, *Rhodopseudomonas*, *Rhodospirillum*, *Roseibium*, *Roseobacter*, *Roseomonas*, *Roseovarius*, *Ruegeria*, *Sagittula*, *Silicibacter*, *Sphingobium*, *Sphingomonas*, *Sphingopyxis*, *Starkeya*, *Sulfitobacter*, *Thalassibium*,
15 *Xanthobacter*, *Zymomonas*, *Agrobacterium*, *Rhizobium*, *Sinorhizobium*, *Anaplasma*, *Ehrlichia*, *Neorickettsia*, *Orientia*, *Rickettsia*, *Wolbachia*, *Bordetella*, *Burkholderia*, *Cupriavidus*, *Taiwanensis*, *Lautropia*, *Limnobacter*, *Polynucleobacter*, *Ralstonia*, *Chromobacterium*, *Eikenella* *corrodens*, *Basfia*, *Kingella*, *Laribacter*, *Lutiella*, *Neisseria*, *Simonsiella*, *Achromobacter*, *Acidovorax*, *Alicyclophilus*, *Aromatoleum*, *Azoarcus*, *Comamonas*, *Dechloromonas*, *Delftia*,
20 *Gallionella*, *Herbaspirillum*, *Hermineimonas*, *Hylemonella*, *Janthinobacterium*, *Leptothrix*, *Methylbium*, *Methylobacillus*, *Methylophilales*, *Methyloversatilis*, *Methylovorus*, *Nitrosomonas*, *Nitrosospora*, *Oxalobacter*, *Parasutterella*, *Polaromonas*, *Polaromonas*, *Pusillimonas*, *Rhodoferax*, *Rubrivivax*, *Sideroxydans*, *Sutterella wadsworthensis*, *Taylorella*, *Thauera*, *Thiobacillus*, *Thiomonas*, *Variovorax*, *Verminephrobacter*, *Anaeromyxobacter*, *Bdellovibrio*
25 *bacteriovorus*, *Bilophila*, *Desulfarculus*, *Desulfatibacillum*, *Desulfobacca*, *Desulfobacterium*, *Desulfobulbus*, *Desulfococcus*, *Desulfohalobium*, *Desulfitobacterium*, *Desulfomicrobium*, *Desulfonatronospora*, *Desulfotalea*, *Desulfovibrio*, *Desulfuromonas*, *Geobacter*, *Haliangium*, *Hipaea*, *Lawsonia*, *Myxococcus*, *Pelobacter*, *Plesiocystis*, *Sorangium*, *Stigmatella*, *Syntrophobacter*, *Syntrophus*, *Arcobacter*, *Caminibacter*, *Campylobacter*, *Helicobacter*,
30 *Nitratifactor*, *Nitratiruptor*, *Sulfuricurvum*, *Sulfurimonas*, *Sulfurospirillum*, *Sulfurovum*, *Wolinella*, *Buchnera*, *Blochmannia*, *Hamiltonella*, *Regiella*, *Riesia*, *Citrobacter*, *Cronobacter*, *Dickeya*, *Edwardsiella*, *Enterobacter*, *Erwinia*, *Escherichia*, *Klebsiella*, *Pantoea*, *Pectobacterium*, *Proteus*, *Providencia*, *Rahnella*, *Salmonella*, *Serratia*, *Shigella*, *Sodalis*, *Wigglesworthia*, *Glossina*, *Xenorhabdus*, *Yersinia*, *Acidithiobacillus*, *Acinetobacter*, *Aeromonas*, *Alcanivorax*,

- Alkalilimnicola*, *Allochromatium*, *Alteromonadales*, *Alteromonas*, *Baumannia*, *Beggiatoa*, *Bermanella*, *Carsonella*, *Ruthia*, *Vesicomyosocius*, *Cardiobacterium*, *Chromohalobacter*, *Colwellia*, *Congregibacter*, *Coxiella*, *Dichelobacter*, *Endoriftia*, *Enhydrobacter*, *Ferrimonas*, *Francisella*, *Glaciecola*, *Hahella*, *Halomonas*, *Halorhodospira*, *Halothiobacillus*, *Idiomarina*,
5 *Kangiella*, *Legionella*, *Marinobacter*, *Marinomonas*, *Methylobacter*, *Methylococcus*, *Methylomicrobium*, *Methylophaga*, *Moraxella*, *Moritella*, *Neptuniibacter*, *Nitrococcus*, *Pseudoalteromonas*, *Psychrobacter*, *Psychromonas*, *Reinekea*, *Rickettsiella*, *Saccharophagus*, *Shewanella*, *Succinatimonas*, *Teredinibacter*, *Thioalkalimicrobium*, *Thioalkalivibrio*, *Thiomicrospira*, *Tolumonas*, *Vibrionales*, *Actinobacillus*, *Aggregatibacter*, *Gallibacterium*,
10 *Haemophilus*, *Histophilus*, *Mannheimia*, *Pasteurella*, *Azotobacter*, *Cellvibrio*, *Pseudomonas*, *Aliivibrio*, *Grimontia*, *Photobacterium*, *Photobacterium*, *Vibrio*, *Pseudoxanthomonas*, *Stenotrophomonas*, *Xanthomonas*, *Xylella*, *Borrelia*, *Brachyspira*, *Leptospira*, *Spirochaeta*, *Treponema*, *Hodgkinia*, *Puniceispirillum*, *Liberibacter*, *Pelagibacter*, *Odyssella* and *Accumulibacter*, in particular *B. subtilis*, *B. megaterium*, *C. glutamicum*, *E. coli*, *Pseudomonas*
15 *sp.*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas stutzeri*, *Acinetobacter* *sp.*, *Burkholderia* *sp.*, *Burkholderia thailandensis*, cyanobacteria, *Klebsiella* *sp.*, *Klebsiella oxytoca*, *Salmonella* *sp.*, *Rhizobium* *sp.* and *Rhizobium meliloti*. In a particularly preferred embodiment it is an enterobacterium, most preferably *Escherichia coli*.
- 20 It is advantageous if the whole cell catalyst according to the invention, as well as the fatty acid reductase, phosphopantetheinyl transferase and the transaminase, also contains an alanine dehydrogenase in order to regenerate from inorganic nitrogen-containing molecules the alanine consumed by the transaminase during the amination of the ω -oxo fatty acid. In a preferred embodiment, the term "alanine dehydrogenase", as used herein, is understood to mean an
25 enzyme which catalyses the conversion of L-alanine with consumption of water and NAD^+ to pyruvate, ammonia and NADH and the reverse reaction. In a particularly preferred embodiment, the alanine dehydrogenase is selected from the group of alanine dehydrogenases which include the amino acid sequence of the alanine dehydrogenase from *Bacillus subtilis* (database code L20916), *Rhizobium leguminosarum* (database code CP001622), *Vibrio proteolyticus*
30 (database code AF070716), *Mycobacterium tuberculosis* (database code X63069), *Enterobacter aerogenes* (database code AB013821), EGR93259.1, YP_003654745.1, YP_003651439.1, YP_003637111.1, YP_003631815.1, YP_001327051.1, YP_001262560.1, YP_886996.1, YP_882850.1, YP_704410.1, YP_703508.1, ZP_08624689.1, YP_001230376.1, P17557.1, P17556.1, CCB94892.1, CCB73698.1, YP_001168635.1, YP_004668736.1,

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 5 YP_004095847.1, YP_003338282.1, YP_003337256.1, YP_355846.1, YP_253131.1,
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 10 YP_575010.1, YP_477594.1, YP_474564.1, YP_130399.1, YP_129373.1, YP_123314.1,
 NP_810467.1, NP_646469.1, NP_626044.1, NP_391071.1, ZP_08086822.1, ZP_08084776.1,
 ZP_08083119.1, ZP_08020768.1, ZP_08013590.1, ZP_08011832.1, YP_003783744.1,
 YP_002781576.1, YP_002780533.1, ZP_02195873.1, NP_797482.1, ZP_07645051.1,
 ZP_07643260.1, ZP_06611917.1, AAT40119.1, ZP_07864946.1, YP_004068409.1,
 15 YP_002796203.1, YP_002774420.1, YP_003600348.1, YP_003599946.1, YP_003565624.1,
 YP_003565223.1, YP_335198.1, YP_423850.1, YP_155059.1, ZP_07843538.1,
 ZP_07841226.1, ZP_06928932.1, ZP_05692073.1, ZP_05687006.1, ZP_04867480.1,
 YP_775531.1, CBE70214.1, ZP_07721182.1, ZP_04302850.1, ZP_04298961.1,
 ZP_04287684.1, ZP_04277177.1, ZP_04248389.1, ZP_04235899.1, ZP_02159718.1,
 20 ZP_02152178.1, YP_003974610.1, YP_003546595.1, YP_002317127.1, ZP_07313778.1,
 ZP_07302778.1, ZP_07298850.1, CBK69442.1, YP_003413835.1, YP_003595089.1,
 ZP_06807811.1, YP_003582455.1, YP_003464731.1, YP_003496397.1, YP_003421918.1,
 CBL07274.1, CBK64956.1, YP_003508515.1, AAL87460.1, AAC23579.1, AAC23578.1,
 AAC23577.1, ACU78652.1, YP_003471439.1, YP_003452777.1, ZP_06384971.1,
 25 ACY25368.1, ABC26869.1, AAP44334.1, EEZ80018.1, ZP_05110458.1, 1PJB_A,
 ZP_04717201.1, ZP_04689103.1, CAO90307.1, CAM75354.1, CAA44791.1, BAA77513.1,
 EGR96638.1, EGL90046.1, YP_004510847.1, ZP_08450330.1, YP_003387804.1,
 YP_003058152.1, EFS74272.1, EFS67128.1, ZP_06844564.1, YP_826658.1,
 YP_001195249.1, YP_003095978.1, YP_469292.1, YP_004442054.1, YP_004461174.1,
 30 YP_004055616.1, YP_003576656.1, YP_003094537.1, YP_001295973.1, AEE71143.1,
 YP_004447480.1, YP_003761844.1, YP_040853.1, YP_003154888.1, YP_003142045.1,
 YP_002280953.1, NP_371963.1, NP_422368.1, EGC98966.1, EGC76398.1, YP_004263661.1,
 YP_004252039.1, YP_679036.1, YP_499973.1, ZP_08054972.1, ZP_08053009.1,
 ZP_04067276.1, ZP_03968868.1, ZP_03963857.1, ZP_03933079.1, ZP_03497046.1,

ZP_06668924.1, ZP_06667106.1, ZP_06324464.1, ZP_06196777.1, ZP_05114159.1, ZP_05083968.1, ZP_05070370.1, ZP_05030022.1, ZP_04673064.1, ZP_03517011.1, ZP_03505783.1, XP_001310698.1, ABK27691.1 and CAB59281.2 and variants thereof. For the reaction catalysed by the alanine dehydrogenase, the presence not only of pyruvate, which is
 5 formed as part of the primary metabolism by any cell possible as a whole cell catalyst, but also of ammonium, is necessary. The latter is typically provided in the form of inorganic nitrogen salts, for example ammonium salts, nitrates or the like. Preferably, an ammonium salt, e.g. ammonium chloride, is added to the aqueous reaction medium.

10 Furthermore, it is advantageous if the whole cell catalyst according to the invention expresses an alkane hydroxylase and optionally further enzymes essential for the activity of the alkane hydroxylase, in particular for the case that a fatty acid with an oxidation level at the terminal ω carbon atom which lies below the oxidation level of the aldehyde is used as substrate for the production of the ω -amino fatty acid. The alkane hydroxylase and/or an additionally expressed
 15 alcohol dehydrogenase then oxidize the terminal carbon atom to the aldehyde group, which can then be aminated by the transaminase. In a preferred embodiment, the term "alkane hydroxylase", as used herein, is understood to mean an enzyme which catalyses the hydroxylation of unsubstituted linear alkyl residues containing at least six, preferably twelve hydrocarbon residues.

20

As alkane hydroxylases, many oxidation systems are suitable according to the invention, such as are inter alia described in PCT/EP2008/067447. In a preferred embodiment, the alkane hydroxylase is a cytochrome P450 monooxygenase of the CYP153 family. In a preferred embodiment, the term "cytochrome P450 monooxygenase of the CYP153 family" is understood
 25 to mean a cytosolic oxidase which is part of a 3-component system which further contains a ferredoxin and a ferredoxin reductase, with an alkane binding site and the ability to hydroxylate alkanes. In a particularly preferred embodiment, it is an enzyme which has up to at least 80, preferably 90, most preferably 95 or 99 percent sequence identity to the cytochrome P450 monooxygenase of the CYP153 family from *Alcanivorax borkumensis* SK2 (database code
 30 YP_691921) or an enzyme, which contains a polypeptide sequence which has at least 80, preferably 90, most preferably 95 or 99 percent sequence identity to the cytochrome P450 monooxygenase of the CYP153 family from *Alcanivorax borkumensis* SK2 (database code YP_691921) and moreover has alkane hydroxylase activity. Here, as throughout in this application, the said database codes here are based on the NCBI (National Center for

Biotechnology Information, Bethesda, USA) databases, more precisely the version available online on 21 November 2012. In a preferred embodiment, the term "cytochrome P450 monooxygenase of the CYP153 family" is understood to mean a non-membrane-bound oxidase which includes a binding site for alkanes, unsubstituted linear alkyl residues including at least five, preferably twelve hydrocarbon residues or singly hydroxylated alkanes and the polypeptide chain whereof contains the motif LL(I/L)(V/I)GGNDTTRN. In a preferred embodiment, a "cytochrome P450 monooxygenase of the CYP153 family", as used herein, is a cytochrome P450 monooxygenase of the CYP153 family from *Alcanivorax borkumensis* SK2 (database code YP_691921) or a variant thereof, which preferably has alkane hydroxylase activity.

For the optimal supply of the cytochrome P450 monooxygenase of the CYP153 family with electrons from the reducing agent, preferably NADH, it is preferable that the cell expresses the monooxygenase together with ferredoxin reductase functionally interacting with it and ferredoxin functionally interacting with it. Here these can be isolated polypeptides or polypeptides co-expressed during the use of a whole cell catalyst or polypeptides N- or C-terminally fused with the cytochrome P450 monooxygenase of the CYP153 family. Whether a ferredoxin reductase or a ferredoxin with a given cytochrome P450 monooxygenase of the CYP153 family functionally interact with one another can easily be determined by those skilled in the art by whether the reducing agent is oxidized more efficiently in the presence of an alkane substrate and the three polypeptides than in the case where at least one of the three is lacking. Alternatively, the enzyme test described by Scheps, D., Malca, H., Hoffmann, B., Nestl, B. M., and Hauer, B. (2011) *Org. Biomol. Chem.*, 9, 6727 can be used, which in the case of functionally interacting polypeptides shows a marked increase in the reaction rate. In a particularly preferred embodiment, the cytochrome P450 monooxygenase of the CYP153 family, the ferredoxin and the ferredoxin reductase come from the same organism. In a particularly preferred embodiment, they are the ferredoxin reductase from *Alcanivorax borkumensis* SK2 (database code YP_691923) or a variant thereof, the ferredoxin from *Alcanivorax borkumensis* SK2 (database code YP_691920) or a variant thereof and the cytochrome P450 monooxygenase of the CYP153 family from *Alcanivorax borkumensis* SK2 (database code YP_691921) or a variant thereof.

In a further preferred embodiment, the alkane hydroxylase is an AlkB monooxygenase. AlkB is an oxidoreductase which first became known from the AlkBGT system from *Pseudomonas putida* Gpo1, which is dependent on two further polypeptides, AlkG and AlkT. AlkT is

characterized as an FAD-dependent rubredoxin reductase, which passes electrons from NADH on to AlkG. AlkG is a rubredoxin, an iron-containing redox protein, which functions as a direct electron donor for AlkB. In a preferred embodiment, the term "AlkB monooxygenase" is understood to mean a polypeptide with a sequence homology of at least, stated in order of increasing preference, 75, 80, 85, 90, 92, 94, 96, 98 or 99% to the sequence of the AlkB from *Pseudomonas putida* Gpo1 (database code: CAB54050.1; like all others used in the application, this database code derives from the state of the art, namely from the NCBI Database, more precisely the release available online on 15 October 2012) with the ability to oxidize alkanes. In a particularly preferred embodiment, the AlkB monooxygenase is an alkane-oxidizing oxidoreductase functionally interacting with the AlkG (CAB54052.1) and AlkT (CAB54063.1) polypeptides from *Pseudomonas putida* Gpo1. For the optimal supply of the AlkB alkane hydroxylase with electrons, it is preferable that the cell expresses the monooxygenase together with auxiliary proteins functionally interacting with it, preferably AlkG and/or AlkT or respective variants thereof, where in a particularly preferred embodiment these are once again AlkG (CAB54052.1) and AlkT (CAB54063.1) polypeptides from *Pseudomonas putida* Gpo1.

In the use of a whole cell catalyst, the problem can arise that a substrate must be brought into contact with an enzyme located within the cell, so that the desired reaction occurs. In the case of long-chain alkanes and derivatives thereof it is preferable that the whole cell catalyst contains a polypeptide of the AlkL family. AlkL is a membrane protein from *Pseudomonas putida*, which can import long-chain fatty acids and derivatives thereof into bacterial cells. In a preferred embodiment, a "polypeptide of the AlkL family", as used herein, is a polypeptide which over a length of 230 consecutive amino acids has at least 80, preferably 90, still more preferably 90% sequence identity to AlkL from *Pseudomonas putida* (database code CAB69081) or a variant of AlkL from *Pseudomonas putida* and preferably the ability to promote the importation of long-chain alkanes into the interior of a cell. In a further embodiment, a "polypeptide of the AlkL family", as used herein, is a polypeptide located in the outer membrane of a Gram-negative bacterium, which has the sequence motif DXWAPAXQ(V/A)GXR, wherein X represents a proteinogenic amino acid, and preferably additionally is AlkL from *Pseudomonas putida* (database code CAB69081) or a variant thereof. Examples of members of the AlkL family include AlkL from *Pseudomonas putida* (database code CAB69081), *Marinobacter aquaeolei* VT8 (database code YP_957722), *Oceanicaulis alexandrii* HTCC2633 (database code ZP_00953584), *Marinobacter manganoxydans* Mnl7-9 (database code ZP_09158756),

Caulobacter sp. K31 (database code YP_001672217), *Pseudomonas oleovorans* (database code Q00595) and variants thereof.

- 5 The teaching of the present invention can be implemented not only with use of macromolecules with the exact amino acid or nucleic acid sequence, to which reference is made herein, or not only with use of a cell with decreased activity relative to the respective wild type of a polypeptide with the exact amino acid sequence to which reference is made herein, but also with use of a variant of such macromolecules or of a cell with a decreased activity, relative to the respective
- 10 wild type of the respective cell, of a variant of the polypeptide, which can be obtained by deletion, addition or substitution of one or more than one amino acid or nucleic acid. In a preferred embodiment, the term "variant" of a nucleic acid sequence or amino acid sequence, used below equivalently and exchangeably with the term "homolog", as used herein, means another nucleic acid or amino acid sequence, which includes or is a sequence which with
- 15 regard to the corresponding original wild type nucleic acid or amino acid sequence has a homology, here used equivalently with identity, of 70, 75, 80, 85, 90, 92, 94, 96, 98, 99% or more percent, wherein preferably amino acids other than those forming the catalytically active centre or essential for the structure or folding are deleted or substituted or such are only conservatively substituted, for example a glutamate instead of an aspartate or a leucine instead
- 20 of a valine. The state of the art describes algorithms which can be used to calculate the degree of homology of two sequences, e.g. Arthur Lesk (2008), Introduction to bioinformatics, 3rd edition. In a further more preferred embodiment of the present invention, the variant of an amino acid or nucleic acid sequence, preferably in addition to the aforesaid sequence homology, essentially has the same enzymatic activity of the wild type molecule or of the original molecule.
- 25 For example, a variant of a polypeptide enzymatically active as a protease has the same or essentially the same proteolytic activity as the polypeptide enzyme, i.e. the ability to catalyse the hydrolysis of a peptide bond. In a particular embodiment, the term "essentially the same enzymatic activity" means an activity with regard to the substrates of the wild type polypeptide, which lies markedly over the background activity or/and differs by less than 3, more preferably
- 30 2, still more preferably one order of magnitude from the K_M and/or k_{cat} values which the wild type polypeptide displays with regard to the same substrates. In a further preferred embodiment, the term "variant" of a nucleic acid or amino acid sequence comprises at least one active part or fragment of the nucleic acid or amino acid sequence. In a further preferred embodiment, the term "active part", as used herein, means an amino acid sequence or a nucleic acid sequence,
- 35 which has less than the full length of the amino acid sequence or codes for less than the full

length of the amino acid sequence, wherein the amino acid sequence or the encoded amino acid sequence with lesser length than the wild type amino acid sequence has essentially the same enzymatic activity as the wild type polypeptide or a variant thereof, for example as a protease. In a particular embodiment, the term "variant" of a nucleic acid includes a nucleic acid

5 the complementary strand whereof binds to the wild type nucleic acid, preferably under stringent conditions. For those skilled in the art, the stringency of the hybridization reaction is easily determinable and generally depends on the length of the probe, the temperatures during the washing and the salt concentration. In general, longer probes require higher temperatures for the hybridization, whereas shorter probes work with lower temperatures. Whether hybridization

10 takes place depends in general on the ability of the denatured DNA to anneal to complementary strands which are present in their vicinity, and this below the melting temperature. The stringency of hybridization reaction and corresponding conditions are described in more detail in F. M. Ausubel (1995), Current Protocols in Molecular Biology, John Wiley & Sons, Inc. Those skilled in the art find instructions for the identification of DNA sequences by hybridization inter

15 alia in the manual "The DIG system User's Guide for Filter Hybridization" from the firm Boehringer Mannheim GmbH (Mannheim, Germany, 1993) and in Liebl *et al.* (International Journal of Systematic Bacteriology 41: 255-260 (1991)). In a preferred embodiment, the hybridization takes place under stringent conditions, that is, only hybrids wherein probe and target sequence, i.e. the polynucleotides treated with the probe, are at least 70% identical are

20 formed. It is known that the stringency of the hybridization including the washing step is influenced or determined by variation of the buffer composition, the temperature and the salt concentration. In general, the hybridization reaction is performed at relatively low stringency in comparison to the washing steps (Hybaid Hybridisation Guide, Hybaid Limited, Teddington, UK, 1996). For example, a buffer corresponding to 5x SSC buffer at a temperature of ca. 50°C-68°C

25 can be used for the hybridization reaction. During this, probes can also hybridize with polynucleotides which have less than 70% identity to the sequence of the probe. Such hybrids are less stable and are removed by washing under stringent conditions. This can for example be achieved by lowering the salt concentration to 2x SSC and if necessary subsequently 0.5x SSC (The DIG System User's Guide for Filter Hybridization, Boehringer Mannheim, Mannheim,

30 Germany, 1995), wherein a temperature of increasing preference in the order ca. 50°C-68°C, ca. 52°C-68°C, ca. 54°C-68°C, ca. 56°C-68°C, ca. 58°C-68°C, ca. 60°C-68°C, ca. 62°C-68°C, ca. 64°C-68°C, ca. 66°C-68°C is established. Temperature ranges of ca. 64°C-68°C or ca. 66°C-68°C are preferred. It is also possible if necessary to lower the salt concentration to a concentration corresponding to 0.2x SSC or 0.1x SSC. By stepwise raising of the hybridization

temperature in steps of ca. 1-2°C from 50°C to 68°C, polynucleotide fragments can be isolated which for example in order of increasing preference display at least 70% or at least 80% or at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to the sequence of the nucleic acid molecule used. Further instructions on hybridization are obtainable on the market in the form of so-called kits (e.g. DIG Easy Hyb from the firm Roche Diagnostics GmbH, Mannheim, Germany, Catalogue No. 1603558). In a preferred embodiment, the term "variant" of a nucleic acid, as used herein, includes any nucleic acid sequence which in the context of the degeneracy of the genetic code codes for the same amino acid sequence as the original nucleic acid or a variant of this amino acid sequence.

In a preferred embodiment, the cell used according to the invention has activity of at least one enzyme which catalyses one of the reactions of the β -oxidation of fatty acids decreased compared to the wild type thereof, wherein this is preferably an enzyme from the group which comprises fatty acid-CoA ligase, Acyl-CoA dehydrogenase, 2,4-dienoyl-CoA reductase, enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase, a fatty acid importer or variants thereof. The β -oxidation of fatty acids is a widespread metabolic route which allows prokaryotic and eukaryotic organisms alike to oxidize fatty acids and to make the chemical energy contained therein available to the metabolism. In the wider sense, it begins with the uptake of a fatty acid into the cell. There the fatty acid, insofar as the conditions require it, is firstly oxidized at the β position of the CoA fatty acid ester by an acyl-CoA dehydrogenase, in the case of *E. coli* FadE. Alternatively, a similar molecule can also be formed from a doubly unsaturated fatty acid by reduction by means of a 2,4-dienoyl-CoA reductase, in *E. coli* FadH. A multifunctional enzyme, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase, in *E. coli* FadB, then catalyses the hydration with formation of the secondary alcohol and its subsequent oxidation to the ketone. In the last step, a 3-ketoacyl-CoA thiolase, in the case of *E. coli* FadA, catalyses the cleavage of the ketoacyl-CoA with the result that acetyl-CoA and a CoA ester of the fatty acid shortened by two carbon atoms in comparison to the starting molecule are liberated. Provided that it is not also acetyl-CoA, the latter can be fed afresh into the β oxidation cycle and shortened by oxidation. Also involved in the regulation of the β oxidation of fatty acids is FadR, a regulator of the Fad operon, which comprises genes necessary for the degradation of fatty acids, without FadR catalyzing a reaction of the β oxidation. In a preferred embodiment, the term "enzyme which catalyses one of the reactions of the β oxidation of fatty acids" is understood to mean any enzyme which interacts directly with the fatty acid substrate or a molecule formed therefrom on

the pathway to acetyl-CoA, preferably recognizes it as substrate, and catalyses its conversion to a metabolic product lying closer to acetyl-CoA on this degradation pathway, preferably including the fatty acid importer which effects the uptake of the fatty acid into the cell. For example, acyl-CoA dehydrogenase is among these enzymes according to the foregoing definition, since it

5 interacts with the fatty acid CoA ester and catalyses its conversion to the enoyl-CoA, which lies closer to acetyl-CoA on the metabolic pathway of the β oxidation than the fatty acid CoA ester. In a particularly preferred embodiment, the term "enzyme which catalyses one of the reactions of the β oxidation of fatty acids", as used herein, is understood to mean any enzyme from the group which comprises the gene products FadA, FadB, FadD, FadL and FadE from *E. coli*

10 and/or variants or homologues thereof from other organisms. The gene products FadA, FadB, FadD, FadL and FadE from *E. coli*, like also variants and homologues of many other biotechnologically usable organisms and their nucleic acid and polypeptide sequences, are described in the state of the art, for example FadA under access number AP009048.1, FadB under access number BAE77457.1, FadD under access number BAA15609.1, and FadE under

15 access number BAA77891.2. The state of the art discloses many tests which are especially suitable for the measurement of the activity of enzymes which catalyse one of the reactions of the β oxidation of fatty acids, for example in K Kameda & W D Nunn (1981) *J. Biol. Chem.* 256, 5702-5707, H Marrakchi, W E DeWolf, C Quinn, J West, B J Polizzi, C Y So et al. (2003) *Biochem. J.* 370, 1055-1062, Lobo *et al.* (2001) and X Yu, T Liu, F Zhu, and C Khosla (2011)

20 PNAS, *electronic publication before printing*.

For the effectiveness of the whole cell catalyst according to the invention it is advantageous if the substrate to be converted, preferably the fatty acid, ω -hydroxy- or ω -oxo fatty acid, can easily come into contact with the enzymes necessary according to the invention, which are

25 located in the interior of the whole cell catalyst. Hence it is critical that the substrate can reach the interior of the cell. In order to facilitate this, it is preferable that the whole cell catalyst expresses a fatty acid importer, in the case of a bacterial, in particular Gram-negative whole cell catalyst, particularly preferably the fatty acid importer FadL (database code: BAA16205.1, SEQ ID NO: 31) or a variant, preferably in a concentration and with an activity which is increased

30 compared to the activity of the wild type of the corresponding whole cell catalyst. The raising of the activity of a polypeptide compared to the wild type of the cell can be achieved via various routes routinely accessible to those skilled in the art, for example the incorporation of additional copies of the nucleotide sequence coding for the polypeptide functionally linked with a promoter, or the exchange of the natural promoter for a stronger one.

It has been found that the ω -amino fatty acids are produced according to the invention in higher yield and purity when the background of enzymes endogenously expressed in the whole cell catalyst is optimized such that the activity of endogenous enzymes which degrade the educts, intermediate products or products of the method according to the invention or with use of the cell according to the invention, preferably ω -amino fatty acids, on metabolic pathways or modify them in other ways, which lead away from the formation of the desired product, is decreased or switched off. Against this background, it is advantageous if the whole cell catalyst according to the invention is about one cell, which has decreased activity of the esterase BioH [Database code YP_492020.1, SEQ ID NO: 30] or a variant thereof compared to its wild type. Such cells with decreased BioH activity, their production and tests for activity determination are described in the European patent application EP12007663.3.

The whole cell catalyst according to the invention can be used in a method for the conversion of a fatty acid, ω -hydroxy- or ω -oxo fatty acid to the corresponding amine, preferably an ω -amino fatty acid, wherein the fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or the monoester thereof is a compound of the formula (I)



wherein R^1 is selected from the group which comprises -H, -CHO, -OH and $COOR^3$, wherein R^2 and R^3 each and independently of one another are selected from the group which comprises H, methyl, ethyl and propyl, with the proviso that at least one of the residues R^2 and R^3 is H, and wherein A represents an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group with at least four carbon atoms. In a preferred embodiment, A is a structure of the formula $-(CH_2)_n-$, wherein n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30. In a most preferred embodiment, the fatty acid, ω -hydroxy or ω -oxo fatty acid is lauric acid, ω -hydroxy- or ω -oxolauric acid. In a further most preferred embodiment, the fatty acid, ω -hydroxy or ω -oxo fatty acid is hexanoic acid, ω -hydroxy- or ω -oxohexanoic acid. In a further most preferred embodiment the fatty acid, ω -hydroxy or ω -oxo fatty acid is decanoic acid, ω -hydroxy- or ω -oxodecanoic acid.

With regard to the fatty acid as to every chemical compound described in this application, the respective formula stated includes all salts, protonated or deprotonated, of the respective

compound. For example, lauric acid comprises not only the protonated form, but also the salt laurate with all cations, for example sodium laurate.

The process according to the invention requires that the enzymes used for the process according to the invention, optionally provided in the form of the whole cell catalyst according to the invention, are contacted with fatty acid, ω -hydroxy- or ω -oxo fatty acid in an aqueous solution. In a preferred embodiment, the term "contacting", as used herein, is understood to mean that the particular enzyme comes into direct contact with its substrate, without physical barriers such as impermeable membranes or the like being inserted between. In the simplest case, the contacting takes place by addition of the substrate to an aqueous solution in which the enzyme or the whole catalyst is located.

Suitable for the implementation of the teaching according to the invention is a reaction mixture comprising the whole cell catalyst according to the invention in aqueous solution and a fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or a monoester thereof of the formula (I), wherein R^1 is selected from the group which comprises -H, -CHO, -OH and $COOR^3$, wherein R^2 and R^3 each and independently of one another are selected from the group which comprises H, methyl, ethyl and propyl, with the proviso that at least one of the residues R^2 and R^3 is H, wherein A represents an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group with at least four carbon atoms, preferably the formula $-(CH_2)_n-$, and wherein n is at least 4, particularly preferably at least 10. The aqueous solution here must, for example with regard to composition, pH and temperature, be constituted such that it at least for a time supports the viability or at least the catalytic ability of the whole cell catalyst. Many aqueous culture media suitable as the aqueous solution, which are suitable for the maintenance or culturing of cells, in particular biotechnologically important cells, are known to those skilled in the art. These include both complete media such as LB media, minimal media such as M9 media and selective media, for example those which contain a high salt concentration and hence only enable the growth of halophilic or at least halotolerant organisms. In a preferred embodiment, the term "aqueous culture medium", as used herein, is understood to mean a water-based reaction medium, which with regard to all relevant factors, in particular pH, salt content and temperature, is constituted such that it maintains or promotes the viability of cells contained therein, preferably microorganisms, and both aqueous culture medium and also hydrophobic organic phase are present in liquid form. The temperature demands of various biotechnologically important cells can be inferred from microbiology and molecular biology

- textbooks, e.g. Fuchs/Schlegl, 2008. In a preferred embodiment, the pH of the aqueous culture medium at the time of the contacting lies between 4 and 9, more preferably between 4.5 and 8.5, and most preferably between 6.5 and 7.5. In a further preferred embodiment, the temperature lies between 0 and 45°C, more preferably between 15 and 40°C, and most preferably between 20 and 37°C. The reaction mixture is typically contained in a fermenter. Any reaction vessel which can be sterilized, preferably autoclaved, and allows the culturing of the whole cell catalysts, aeration and control of the reaction conditions, for example the oxygen content and the temperature, can function as the fermenter.
- 10 In a preferred embodiment, the reaction mixture in addition to the aqueous solution includes a hydrophobic organic phase. This can comprise an organic solvent and/or a hydrophobic liquid cation exchanger for removal of the ω -amino fatty acid from the aqueous solution. Suitable solvents and cation exchangers are described in EP11191520.3.
- 15 The present invention is further illustrated by the following diagrams and non-limiting examples, from which further features, embodiments, aspects and advantages of the present invention can be inferred.

Example 1**Production of Expression Vectors for Co-Expression of Genes Coding for a Fatty Acid Reductase with *npt* from *Nocardia* sp.**

For the production of vectors for the co-expression of the genes coding for a fatty acid reductase with *npt* (SEQ ID No.1, codes for ABI83656.1) from *Nocardia* sp., which codes for a phosphopantetheinyl transferase, the genes were codon-optimized for expression in *Escherichia coli* and synthesized together with a lacuv5 promoter (SEQ ID No.2) and at the same time a restriction cleavage site was introduced upstream and downstream. The following fatty acid reductase genes were used:

- 10 • MSMEG_2956 from *Mycobacterium smegmatis* (carA, SEQ ID No.3, codes for YP_887275.1)
- MSMEG_5739 from *Mycobacterium smegmatis* (carB, SEQ ID No.4, codes for YP_889972.1)
- FadD9 from *Mycobacterium intracellulare* MOTT-64 (car_Mint, SEQ ID No.5, codes for
- 15 YP_005349252.1)
- MFORT_07381 from *Mycobacterium fortuitum* subsp. *fortuitum* DSM 46621 (car_Mfort, SEQ ID No.6, codes for ZP_11001941.1)
- FadD9 from *Mycobacterium avium* subsp. *paratuberculosis* K-10 (car_Mavi, SEQ ID No.7, codes for NP_959974.1)
- 20 • CPCC7001_1320 from *Cyanobium* sp. PCC 7001 (car_Cs, SEQ ID No.8, codes for ZP_05045132.1)
- MAB_3367 from *Mycobacterium abscessus* ATCC 19977 (car_Mab, SEQ ID No.9, codes for YP_001704097.1)
- FadD9 from *Nocardia brasiliensis* ATCC 700358 (car_nbr, SEQ ID No.10, codes for
- 25 ZP_09839660.1)
- MIP_06852 from *Mycobacterium indicus pranii* MTCC 9506 (car_Mip, SEQ ID No.11, codes for YP_006731697.1)
- nfa20150 from *Nocardia farcinica* IFM 10152 (car_nfa, SEQ ID No.12, codes for YP_118225.1)
- 30 • MUL_1722 from *Mycobacterium ulcerans* Agy99 (car_Mul, SEQ ID No.13, codes for YP_905678.1)
- Saci8_010100039603 from *Streptomyces acidiscabies* 84-104 (car_Sac, SEQ ID No.14, codes for ZP_10456477.1)

- MCOL_V203220 from *Mycobacterium colombiense* CECT 3035 (car_Mcol, SEQ ID No.15, codes for WP_007769435.1)

The DNA fragment synthesized was digested with the restriction endonucleases *NdeI* and *AvrII* and ligated into the correspondingly cleaved vector pSC101 (SEQ ID No.16). The vector pSC101 is a very low copy vector, which mediates tetracycline resistance and has a low copy number of ca. 4 copies per cell. In this way, the following expression vectors could be produced:

- pSC101{Placuv5}[carA_Ms(co_Ec)-npt_Noc(co_Ec)] (SEQ ID No.17)
- pSC101{Placuv5}[carB_Ms(co_Ec)-npt_Noc(co_Ec)] (SEQ ID No.18)
- pSC101{Placuv5}[car_Mint(co_Ec)-npt_Noc(co_Ec)] (SEQ ID No.19)
- 10 • pSC101{Placuv5}[car_Mfort(co_Ec)-npt_Noc(co_Ec)] (SEQ ID No.20)
- pSC101{Placuv5}[car_Mavi(co_Ec)-npt_Noc(co_Ec)] (SEQ ID No.21)
- pSC101{Placuv5}[car_Csp(co_Ec)-npt_Noc(co_Ec)] (SEQ ID No.22)
- pSC101{Placuv5}[car_Mab(co_Ec)-npt_Noc(co_Ec)] (SEQ ID No.23)
- pSC101{Placuv5}[car_nbr(co_Ec)-npt_Noc(co_Ec)] (SEQ ID No.24)
- 15 • pSC101{Placuv5}[car_Mip(co_Ec)-npt_Noc(co_Ec)] (SEQ ID No.25)
- pSC101{Placuv5}[car_nfa(co_Ec)-npt_Noc(co_Ec)] (SEQ ID No.26)
- pSC101{Placuv5}[car_Mul(co_Ec)-npt_Noc(co_Ec)] (SEQ ID No.27)
- pSC101{Placuv5}[car_Sac(co_Ec)-npt_Noc(co_Ec)] (SEQ ID No.28)
- pSC101{Placuv5}[car_Mcol(co_Ec)-npt_Noc(co_Ec)] (SEQ ID No.29)

- 20 For the activation of the fatty acid reductase, the gene product *npt* transfers a phosphopantetheinyl residue from coenzyme A to the fatty acid reductase enzyme.

Example 2

25 **Production of *E. coli* Strains with Deletion in the Gene *bioH* with Intensified Fatty Acid Reductase and Phosphopantetheinyl Transferase Activity for the Production of Aminolauric Acid Methyl Ester from Lauric Acid Methyl Ester**

- For the creation of *E. coli* strains with deletion of the gene *bioH*, which codes for an esterase, and intensified fatty acid reductase activity for the production of aminolauric acid methyl ester, the strain *Escherichia coli* W3110 Δ*bioH* (production: see EP12007663) was transformed with
- 30 the plasmids pBT10_alkL (sequence and production: compare example 1 of WO/2011/131420 and the Seq ID No.8 listed there), pJ294_alaDH_B.s._TA_C.v.(Ct) (compare example 1 of WO/2013/024114 and the SEQ ID No.17 listed there) and one of the vectors created in example 1 for the expression of a fatty acid reductase by electroporation and plated out onto LB agar

plates containing kanamycin (50 µg/ml), ampicillin (100 µg/ml) and tetracycline (5 µg/ml).

Transformants were tested for the presence of the correct plasmid by plasmid preparation and restriction analysis. The following strains were thus created:

- 5 • *E.coli* W3110 ΔbioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}{carA_Ms(co_Ec)-npt_Noc(co_Ec)}
- *E.coli* W3110 ΔbioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}{carB_Ms(co_Ec)-npt_Noc(co_Ec)}
- *E.coli* W3110 ΔbioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}{car_Mint(co_Ec)-npt_Noc(co_Ec)}
- 10 • *E.coli* W3110 ΔbioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}{car_Mfort(co_Ec)-npt_Noc(co_Ec)}
- *E.coli* W3110 ΔbioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}{car_Mavi(co_Ec)-npt_Noc(co_Ec)}
- *E.coli* W3110 ΔbioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}{car_Csp(co_Ec)-npt_Noc(co_Ec)}
- 15 • *E.coli* W3110 ΔbioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}{car_Mab(co_Ec)-npt_Noc(co_Ec)}
- *E.coli* W3110 ΔbioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}{car_nbr(co_Ec)-npt_Noc(co_Ec)}
- 20 • *E.coli* W3110 ΔbioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}{car_Mip(co_Ec)-npt_Noc(co_Ec)}
- *E.coli* W3110 ΔbioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}{car_nfa(co_Ec)-npt_Noc(co_Ec)}
- *E.coli* W3110 ΔbioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}{car_Mul(co_Ec)-npt_Noc(co_Ec)}
- 25 • *E.coli* W3110 ΔbioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}{car_Sac(co_Ec)-npt_Noc(co_Ec)}
- *E.coli* W3110 ΔbioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}{car_Mcol(co_Ec)-npt_Noc(co_Ec)}

30

These strains were used in order to study their capacity for the production of aminolauric acid methyl ester starting from lauric acid methyl ester.

The expression vector pBT10_alkL contains the genes *alkB*, *alkG*, *alkT*, *alkS* and *alkL* from the alk operon of *Pseudomonas putida*. Through the gene products, the oxidation of the substrate lauric acid methyl ester via hydroxylauric acid methyl ester to oxolauric acid methyl ester is catalysed. The vector pJ294_alaDH_B.s._TA_C.v.(Ct) contains the genes *ald* from *Bacillus subtilis* (coding for an alanine dehydrogenase, NP_391071.1) and Cv_2505 from *Chromobacterium violaceum* (coding for an ω -transaminase, NP_901695.1). The gene product Cv_2505 is capable of converting oxolauric acid methyl ester to aminolauric acid methyl ester, and the amino donor alanine needed for this is made available by the gene product *ald* from pyruvate. On the basis of an additionally expressed fatty acid reductase, which was activated by the likewise overexpressed phosphopantetheinyl transferase *npt*, by-products arising in the production of aminolauric acid methyl ester, in particular dodecanedioic acid methyl ester, should be reduced in order to increase the ratio of aminolauric acid methyl ester to dodecanedioic acid methyl ester.

15 Example 3

Production of *E.coli* Strains with Increased Fatty Acid Reductase and Phosphopantetheinyl Transferase Activity for the Production of Aminolauric Acid Methyl Ester from Lauric Acid Methyl Ester

The host strain *E.coli* W3110 used was transformed with the expression vectors pBT10_alkL (sequence and production: compare example 1 of WO/2011/131420 and the Seq ID No.8 listed there), pJ294_alaDH_B.s._TA_C.v.(Ct) (compare example 1 of WO/2013/024114 and the SEQ ID No.17 listed there) and pSC101{Placuv5}[carB_Ms(co_Ec)-npt_Noc(co_Ec)] or pSC101, empty vector, by electroporation and plated out onto LB agar plates containing kanamycin (50 µg/ml), ampicillin (100 µg/ml) and tetracycline (5 µg/ml). Transformants were tested for the presence of the correct plasmid by plasmid preparation and restriction analysis. Thus the strains: *E.coli* W3110 pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}[carB_Ms(co_Ec)-npt_Noc(co_Ec)] and *E.coli* W3110 pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101 were created.

30 Example 4

Production of Aminolauric Acid by *E.coli* Strains with an Expression Vector for a Fatty Acid Reductase Gene and the Gene *npt* from *Nocardia* sp. and Expression Vectors for the Genes *ald* from *Bacillus subtilis*, Cv_2025 from *Chromobacterium violaceum* in

Combination with an Expression Vector for the Genes *alkB*, *alkG*, *alkT* and *alkL* from the *alk* Operon of *Pseudomonas putida*.

The strains created in example 2 and 3 were used in order to investigate their capacity for the production of aminolauric acid methyl ester.

- 5 The biotransformation of lauric acid methyl ester to aminolauric acid methyl ester was carried out in the 8-fold parallel fermentation system of DASGIP. The procedure for this was as follows: For the fermentation, 1 L reactors were used. The pH probes were calibrated by a two-point calibration with standard solutions of pH 4.0 and pH 7.0. The reactors were filled with 300 mL drinking water and autoclaved for 20 mins at 121°C in order to ensure sterility. Next, the pO₂ probes were polarized overnight (for at least 6 hrs) on the DASGIP system. On the next morning, the water was removed under the Clean Bench and replaced by 300 mL high cell density medium containing 100 mg/L ampicillin, 50 mg/L kanamycin and 5 mg/L tetracycline. Subsequently, the pO₂ probes were calibrated with a one-point calibration (stirrer: 400 rpm / aeration: 10 sL/hr air) and the feed, correction medium and induction medium lines cleaned by means of Clean-in-Place. For this the tubes were flushed with 70% ethanol, then with 1 M NaOH, then with sterile demineralized water and finally filled with the respective media.

- The ALS and ALSME producing *E. coli* strains were firstly grown up from the respective cryocultures in LB medium (25 mL in a 100 mL baffle flask) containing 100 mg/L ampicillin overnight at 37°C and 200 rpm for ca. 18 hrs. Next, 2 mL of each of the cultures were inoculated into high density cell medium (glucose 15 g/L (30 mL / L of a separately autoclaved 500 g/L stock solution containing 1% MgSO₄*7H₂O and 2.2% NH₄Cl), (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 2.24 g/L, Ammonium iron citrate solution 17 mL/L of a separately autoclaved 1% stock solution, trace element solution 5 mL/L separately autoclaved stock solution (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)) (25 mL per strain in a 100 mL baffle flask) containing 100 mg/L ampicillin, 50 mg/L kanamycin and 5 mg/L tetracycline and incubated at 37°C/200 rpm for a further 5.5 hrs.
- 30 The reactors were inoculated with an optical density of 0.1 by drawing up an appropriate volume of the preculture into a 5 mL syringe (under sterile conditions) and inoculating the reactors via cannulae through a septum coated with 70% ethanol.

The following standard program was used:

DO Regulator		pH Regulator	
Preset	0%	Preset	0 ml/h
P	0.1	P	5
Ti	300 s	Ti	200 s
Min	0%	Min	0 mL/hr
Max	100%	Max	40 mL/hr

N (Rotation)	from	to	XO ₂ (gas mixture)	from	to	F (Gas flow)	from	to
	0%	30%		0%	100%		15%	80%
Growth and biotrans- formation	400 rpm	1500 rpm	Growth and biotrans- formation	21%	21%	Growth and biotrans- formation	6 sL/hr	72 sL/hr

5

Script	
Trigger exactly	31% DO (1/60 hr)
Induction IPTG	2 hrs after feed start
Feed trigger	50% DO
Feed rate	3 [mL/hr]

The experiment performed can be subdivided into two phases, growth, in which the cells should reach a defined optical density, and the subsequent biotransformation, in which after addition of the substrate lauric acid methyl ester a conversion to aminolauric acid ester by enzymes formed during expression should take place. The pH values were regulated one-sidedly with ammonia (12.5%) to pH 6.8. During growth and biotransformation, the dissolved oxygen (DO) in the culture was regulated at 30% via stirrer rotation rate and aeration rate. The fermentation was carried out as fed-batch, wherein the feed start, 5 g/Lhr glucose feed (500 g/L glucose with 1% MgSO₄·7H₂O and 2.2% NH₄Cl) was triggered via a DO peak. At feed start, the temperature was also lowered from 37°C previously to 30°C. The expression of the transaminase, alanine dehydrogenase and fatty acid reductase was induced 2 hrs after feed start by the automatic addition of IPTG (1 mM). The induction of the alk genes was effected by the manual addition of DCPK (0.025% v/v) 10 hrs after feed start. Before the start of the biotransformation, the optical density of the culture broths was determined.

The start of the biotransformation phase took place 14 hrs after feed start. For this, 150 mL of a mixture of lauric acid methyl ester and the ion exchanger oleic acid (techn. 90%) were added as a batch to the fermentation broth. In order to make an amino group donor available for the transaminase, half an hour before biotransformation start 5 mL of a 3M ammonium sulphate solution was added to the fermentation broth. For sampling, 2 mL of fermentation broth were withdrawn from the vessel and a portion thereof diluted 1/20 in an acetone-HCl mixture ($c(\text{HCl}) = 0.1 \text{ mol/L}$) and extracted. Samples were taken from all reactors at 1 hr, 2 hrs, 3 hrs, 4 hrs, 5 hrs, 7.5 hrs, 10.5 hrs, 19.5 hrs and 21 hrs after the start of the biotransformation. The conversion rates for oxygen (OTR = oxygen transfer rate) and carbon (CTR = carbon transfer rate) were determined during the fermentation via the exhaust gas analysis on the DASGIP systems. The fermentation was ended 21 hrs after the start of the biotransformation. The stirrer, the aeration, the temperature regulation and pH regulation were switched off and the vessel allowed to stand undisturbed for 5-10 minutes.

For the quantification of DDS (C12-dicarboxylic acid), DDSME (C12-dicarboxylic acid methyl ester), LS (lauric acid), LSME (lauric acid methyl ester), HLS (omega-hydroxy lauric acid), HLSME (omega-hydroxy lauric acid methyl ester), OLS (omega-oxo lauric acid), OLSME (omega-oxo lauric acid methyl ester), ALS (omega-amino lauric acid) and ALSME (omega-amino lauric acid methyl ester) in fermentation samples, samples were withdrawn during the culturing. These samples were prepared for the analysis (see LC-ESI/MS²-based quantification of products).

LC-ESI/MS²-based quantification of products

The quantification of ALS, ALSME, DDS, DDSME, LS, LSME, HLS, HLSME, OLS and OLSME in fermentation samples was effected by LC-ESI/MS² on the basis of an external calibration for all analytes (0.1 – 50 mg/L) and with use of the internal standards aminoundecanoic acid (AUD for HLS, DDS, OLS, HLSME, OLSME), d4-ALSME (for ALSME), ¹³C-DDSME (for DDSME), d3-LS (for LS) and d3-LSME (for LSME).

In this, the following instruments are used:

- HPLC System 1260 (Agilent; Böblingen) with autosampler (G1367E), binary pump (G1312B) and column oven (G1316A)
- Mass spectrometer TripleQuad 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 solvent (80% (v/v) ACN, 20% bidist. H_2O (v/v), + 0.1% formic acid) and 100 μL of sample into a 2 mL reaction vessel. The mixture was vortexed for 10 seconds and then centrifuged at ca. 13000 rpm for 5 mins. The clear supernatant was withdrawn with a pipette and analysed after appropriate dilution with diluent (80% (v/v) ACN, 20% bidist. H_2O (v/v), + 0.1% formic acid). 100 μL ISTD were pipetted into each 900 μL sample (10 μL with a sample volume of 90 μL).

- 10 The HPLC separation was effected with the aforesaid column and precolumn. The injection volume is 0.7 μL , the column temperature 50°C, and the flow rate 0.6 mL/min. The mobile phase consists of eluent A (0.1% (v/v) aqueous formic acid) and eluent B (acetonitrile with 0.1% (v/v) formic acid). The following gradient profile was used:

Time [mins]	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

15

The ESI-MS² analysis was effected in positive mode with the following ESI source parameters:

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

The detection and quantification of the compounds ALS, ALSME, DDS, DDSME, HLS, HLSME, OLS, OLSME was effected with the following MRM parameters, wherein in each case one product ion was used as *Qualifier* and one as *Quantifier*.

25

Analyte	Precursor ion [m/z]	Product ion [m/z]	Residence time [ms]	Collision energy [eV]
DDSME	245.2	167.1	25	6
DDSME	245.2	149.1	50	8
HLSME	231.3	181.2	15	2
HLSME	231.3	163.2	25	5
DDS	231.2	213.2	50	0
DDS	231.2	149.1	25	9
ALSME	230.3	198.1	25	10
ALSME	230.3	163.2	15	10
OLSME	229.2	197.2	50	0
OLSME	229.2	161.1	25	5
HLS	217.2	181.2	35	0
HLS	217.2	163.1	20	4
OLS	215.2	161.2	25	0
OLS	215.2	95.2	60	13

The analytes LS and LSME were detected in SIM mode (m/z 201 and 215).

- 5 It was shown that the strains are capable of producing aminolauric acid methyl ester from lauric acid methyl ester via the intermediate stages hydroxylauric acid methyl ester and oxolauric acid methyl ester and at the same time of reducing by-products formed such as dodecanedioic acid methyl ester and dodecanedioic acid with the aid of a fatty acid reductase activity and thus again introducing them into the metabolism for the formation of aminolauric acid methyl ester
- 10 (Tab. 1 and 2). It was further shown that through the introduction of the various fatty acid reductases the ratio of desired products (aminolauric acid methyl ester and aminolauric acid) to by-products (dodecanedioic acid methyl ester and dodecanedioic acid) was increased (Tab. 1 and 2). It was also shown that through the introduction of the various fatty acid reductases, the final product concentration of aminolauric acid methyl ester was increased (Tab. 1 and 2).
- 15 Finally it was shown that through the introduction of a fatty acid reductase the space-time yield of the aminolauric acid methyl ester formation (measured between 1 and 19 hrs after the start of the biotransformation) was increased and the related product-specific glucose consumption decreased (Tab. 2).
- 20 Tab. 1: Product and by-product formation of the strains generated in example 2 with overexpressed fatty acid reductase and the gene *npt* from *Nocardia* sp. relative to the strain without fatty acid reductase activity.

Strain	Relative ALSME formation [%]	Relative DDSME formation [%]	ALSME/DDSME ratio
<i>E.coli</i> W3110 Δ bioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct)	100	100	4.5
<i>E.coli</i> W3110 Δ bioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}[carA_Ms(co_Ec)- npt_Noc(co_Ec)]	103	12	38.8
<i>E.coli</i> W3110 Δ bioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}[carB_Ms(co_Ec)- npt_Noc(co_Ec)]	105	26	18.9
<i>E.coli</i> W3110 Δ bioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}[car_Mint(co_Ec)- npt_Noc(co_Ec)]	106	27	17.7
<i>E.coli</i> W3110 Δ bioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}[car_Mfort(co_Ec)- npt_Noc(co_Ec)]	103	37	12.3
<i>E.coli</i> W3110 Δ bioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}[car_Mavi(co_Ec)- npt_Noc(co_Ec)]	112	21	23.3
<i>E.coli</i> W3110 Δ bioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}[car_Csp(co_Ec)- npt_Noc(co_Ec)]	101	87	5.2
<i>E.coli</i> W3110 Δ bioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}[car_Mab(co_Ec)- npt_Noc(co_Ec)]	111	20	24.9
<i>E.coli</i> W3110 Δ bioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}[car_nbr(co_Ec)- npt_Noc(co_Ec)]	109	24	20.4
<i>E.coli</i> W3110 Δ bioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}[car_Mip(co_Ec)- npt_Noc(co_Ec)]	101	66	6.8
<i>E.coli</i> W3110 Δ bioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}[car_nfa(co_Ec)- npt_Noc(co_Ec)]	110	25	19.6
<i>E.coli</i> W3110 Δ bioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}[car_Mul(co_Ec)- npt_Noc(co_Ec)]	107	35	13.6

<i>E.coli</i> W3110 Δ bioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}[car_Sac(co_Ec)- npt_Noc(co_Ec)]	102	87	5.3
<i>E.coli</i> W3110 Δ bioH pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}[car_Mcol(co_Ec)- npt_Noc(co_Ec)]	107	31	15.4

Tab. 2: Product and by-product formation, product formation rate and yield by the strain generated in example 3, which overexpresses a fatty acid reductase and the gene *npt* from *Nocardia* sp. relative to the strain without fatty acid reductase activity.

Strain	Relative ALS(ME) formation [%]	Relative DDS(ME) formation [%]	ALS(ME)/ DDS(ME) ratio	Relative space- time yield [%]	Relative yield ALS(ME)/ Glucose [%]
<i>E.coli</i> W3110 pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101{Placuv5}[carB_Ms(co_Ec)- npt_Noc(co_Ec)]	121	40	37.02	133	123
<i>E.coli</i> W3110 pBT10_alkL / pJ294_alaDH_B.s._TA_C.v.(Ct) / pSC101	100	100	12.15	100	100

Claims

1. A whole cell catalyst which comprises a transaminase and one selected from the group consisting of
 - 1) an α -dioxygenase and
 - 2) a combination of 2a) a recombinant fatty acid reductase and 2b) a phosphopantetheinyl transferase,
 wherein:
 - the phosphopantetheinyl transferase phospho-pantetheinylates the fatty acid reductase, thereby forming a phosphopantetheinylated fatty acid reductase, the fatty acid reductase catalyzes the conversion of an ω -carboxy acid to a corresponding ω -oxo fatty acid,
 - at least one of the phosphopantetheinyl transferase and transaminase is recombinant,
 - when the catalyst comprises the transaminase and the α -dioxygenase, the transaminase is overexpressed, and when the catalyst comprises the transaminase and the combination of 2a) a recombinant fatty acid reductase and 2b) a phosphopantetheinyl transferase, the transaminase and/or the phosphopantetheinyl transferase is overexpressed.
2. The whole cell catalyst according to claim 1, wherein the whole cell catalyst additionally comprises a recombinant amino acid dehydrogenase.
3. The whole cell catalyst according to claim 1, wherein the whole cell catalyst additionally comprises an alkane hydroxylase.
4. The whole cell catalyst according to claim 1, wherein the whole cell catalyst additionally comprises a polypeptide of the AlkL family.
5. The whole cell catalyst according to claim 1, wherein the whole cell catalyst additionally comprises an alcohol dehydrogenase.
6. The whole cell catalyst according to claim 1, wherein an activity of at least one enzyme of the cell involved in β -oxidation, selected from the group consisting of a fatty acid-CoA ligase, an acyl-CoA dehydrogenase, a 2,4-dienoyl-CoA reductase, an enoyl-CoA

hydratase, a 3-ketoacyl-CoA thiolase, and a fatty acid importer, is decreased compared to the wild type of the whole cell of the catalyst.

7. The whole cell catalyst according to claim 1, wherein an esterase activity of BioH, or a variant of BioH having at least 70% amino acid sequence identity to SEQ ID NO: 30 and essentially the same esterase activity as BioH, in the whole cell is decreased compared to a wild type of the whole cell of the catalyst.

8. The whole cell catalyst according to claim 1, wherein a fatty acid importing activity of FadL, or a variant of FadL having at least 70% amino acid sequence identity to SEQ ID NO: 31 and essentially the same fatty acid importing activity as FadL, is increased in the whole cell of the catalyst compared to a wild type of the whole cell of the catalyst.

9. A method for preparing an amine, the method comprising:

a) oxidizing a starting material comprising a fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or monoester thereof by contacting the starting material with an alkane hydroxylase, an alcohol dehydrogenase, or a mixture thereof to obtain an oxidation product;

b) contacting the oxidation product with a phosphopantetheinylated fatty acid reductase or an α -dioxygenase to obtain an aldehyde; and

c) contacting the aldehyde with a transaminase to obtain the amine,

wherein the phosphopantetheinylated fatty acid reductase or the α -dioxygenase in addition to the transaminase is provided in the form of the whole cell catalyst according to claim 1.

10. The method according to claim 9, wherein in c) an amino acid acts as an amine donor, and an amino acid dehydrogenase regenerates the amino acid after a transamination reaction.

11. The method according to claim 9, wherein the fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or the monoester thereof is a compound of the formula (I)



5

wherein

R^1 is -H, -CHO, -OH and $COOR^3$,

R^2 and R^3 are each independently H, methyl, ethyl or propyl,

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with the proviso that at least one of the residues R^2 and R^3 is H, and

A is an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group with at least four carbon atoms.

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12. The method according to claim 11, wherein A is $-(CH_2)_n-$, and n is at least 4.

13. A method for preparing an amine, the method comprising:

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a) oxidizing a starting material comprising a fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or monoester thereof by contacting the starting material with an alkane hydroxylase, an alcohol dehydrogenase, or a mixture thereof to obtain an oxidation product;

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b) contacting the oxidation product with a phosphopantetheinylated fatty acid reductase or an α -dioxygenase to obtain an aldehyde; and

c) contacting the aldehyde with a transaminase to obtain the amine;

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wherein at least one enzyme of a), b) and c) is present in the form of the whole cell catalyst according to claim 1.

14. The method according to claim 13, wherein in c) an amino acid acts as an amine donor, and an amino acid dehydrogenase regenerates the amino acid after a transamination reaction.

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15. The method according to claim 13, wherein the fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or the monoester thereof is a compound of formula (I)



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wherein

R^1 is -H, -CHO, -OH and $COOR^3$,

R^2 and R^3 are each independently H, methyl, ethyl and propyl,

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with the proviso that at least one of the residues R^2 and R^3 is H, and

A is an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group with at least four carbon atoms.

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16. The method according to claim 15, wherein A is - $(CH_2)_n$ -, and n is at least 4.

17. A reaction mixture comprising:

water;

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the whole cell catalyst according to claim 1; and

a fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or a monoester thereof of the formula (I)



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wherein

R^1 is -H, -CHO, -OH and $COOR^3$,

R^2 and R^3 are each independently H, methyl, ethyl or propyl, with the proviso that at least one of the residues R^2 and R^3 is H, and

5 A is an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group with at least four carbon atoms.

18. The whole cell catalyst according to claim 1, comprising the transaminase and 1) the α -dioxxygenase.
- 10 19. The whole cell catalyst according to claim 1, comprising the transaminase and 2) the combination of 2a) a recombinant fatty acid reductase and 2b) a phosphopatetheinyl transferase.