

***Candida tropicalis* cells and use thereof**

Field of the invention

The invention relates to genetically engineered *Candida tropicalis* cells, use thereof and a method of production of ω -hydroxycarboxylic acids and ω -hydroxycarboxylic acid esters.

Prior art

Owing to its ability to form dicarboxylic acids from alkanes, *Candida tropicalis* is a well-characterized ascomycete.

WO91/006660 describes *Candida tropicalis* cells that are completely inhibited in β -oxidation through interruption of the POX4 and/or POX5 genes, and achieve increased yields of α,ω -dicarboxylic acids.

WO00/020566 describes cytochrome P450 monooxygenases and NADPH cytochrome P450 oxidoreductases from *Candida tropicalis* and use thereof for influencing ω -hydroxylation, the first step in ω -oxidation.

WO03/089610 describes enzymes from *Candida tropicalis* which catalyse the second step of ω -oxidation, the conversion of a fatty alcohol to an aldehyde, and use thereof for improved production of dicarboxylic acids.

The cells and methods described so far are not suitable for the production of ω -hydroxycarboxylic acids or their esters, as the ω -hydroxycarboxylic acids are always only present as a short-lived intermediate and are immediately metabolized further.

ω -Hydroxycarboxylic acids and their esters are economically important compounds as precursors of polymers, and this forms the basis of the commercial usability of the present invention.

The task of the invention was to find a way of preparing ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters by fermentation in sufficient amounts, in particular in the medium surrounding the cells.

Description of the invention

It was found, surprisingly, that the cells described hereunder make a contribution to solution of this task.

The object of the present invention is therefore a cell as described in claim 1.

Another object of the invention is the use of the cell according to the invention and a method of production of ω -hydroxycarboxylic acids and ω -hydroxycarboxylic acid esters using the cells according to the invention.

Advantages of the invention are the gentle conversion of the educt used to the ω -hydroxycarboxylic acids and corresponding esters and a high specificity of the method and an associated high yield based on the educt used.

One object of the present invention is a *Candida tropicalis* cell, in particular one from the strain ATCC 20336, which is characterized in that the cell has, compared with its wild type, a reduced activity of at least one of the enzymes that are encoded by the intron-free nucleic acid sequences selected from the two group comprising

A) Seq ID No. 1, Seq ID No. 3, Seq ID No. 5, Seq ID No. 7, Seq ID No. 9, Seq ID No. 11, Seq ID No. 13, Seq ID No. 15, Seq ID No. 17, Seq ID No. 19, Seq ID No. 21, Seq ID No. 23, Seq ID No. 25, Seq ID No. 27, Seq ID No. 29, Seq ID No. 31, Seq ID No. 33, Seq ID No. 35, Seq ID No. 37, Seq ID No. 39, Seq ID No. 41, Seq ID No. 43, Seq ID No. 45, Seq ID No. 47, Seq ID No. 49, Seq ID No. 51, Seq ID No. 53, Seq ID No. 55, Seq ID No. 57, Seq ID No. 59, Seq ID No. 61, Seq ID No. 63, Seq ID No. 65 and Seq ID No. 67; in particular Seq ID No. 1, Seq ID No. 3, Seq ID No. 5, Seq ID No. 7, Seq ID No. 9, Seq ID No. 11, Seq ID No. 13, Seq ID No. 15, Seq ID No. 17, Seq ID No. 19, Seq ID No. 21, Seq ID No. 23, Seq ID No. 25, Seq ID No. 27, Seq ID No. 29, Seq ID No. 31, Seq ID No. 33, Seq ID No. 35, Seq ID No. 37, Seq ID No. 39, Seq ID No. 41, Seq ID No. 43, Seq ID No. 45, Seq ID No. 47, Seq ID No. 49 and Seq ID No. 51; quite especially Seq ID No. 1, Seq ID No. 3, Seq ID No. 5, Seq ID No. 7, Seq ID No. 9, Seq ID No. 11, Seq ID No. 13, Seq ID No. 15, Seq ID No. 17, Seq ID No. 19, Seq ID No. 21, Seq ID No. 23, Seq ID No. 25 and Seq ID No. 27,

B) a sequence that is identical to at least 80%, especially preferably to at least 90%, even more preferably to at least 95% and most preferably to at least 99% to one of the sequences Seq ID No. 1, Seq ID No. 3, Seq ID No. 5, Seq ID No. 7, Seq ID No. 9, Seq ID No. 11, Seq ID No. 13, Seq ID No. 15, Seq ID No. 17, Seq ID No. 19, Seq ID No. 21, Seq ID No. 23, Seq ID No. 25, Seq ID No. 27, Seq ID No. 29, Seq ID No. 31, Seq ID No. 33, Seq ID No. 35, Seq ID No. 37, Seq ID No. 39, Seq ID No. 41, Seq ID No. 43, Seq ID No. 45, Seq ID No. 47, Seq ID No. 49, Seq ID No. 51, Seq ID No. 53, Seq ID No. 55, Seq ID No. 57, Seq ID No. 59, Seq ID No. 61, Seq ID No. 63, Seq ID No. 65 and Seq ID No. 67; in particular to Seq ID No. 1, Seq ID No. 3, Seq ID No. 5, Seq ID No. 7, Seq ID No. 9, Seq ID No. 11, Seq ID No. 13, Seq ID No. 15, Seq ID No. 17, Seq ID No. 19, Seq ID No. 21, Seq ID No. 23, Seq ID

No. 25, Seq ID No. 27, Seq ID No. 29, Seq ID No. 31, Seq ID No. 33, Seq ID No. 35, Seq ID No. 37, Seq ID No. 39, Seq ID No. 41, Seq ID No. 43, Seq ID No. 45, Seq ID No. 47, Seq ID No. 49 and Seq ID No. 51; quite especially to Seq ID No. 1, Seq ID No. 3, Seq ID No. 5, Seq ID No. 7, Seq ID No. 9, Seq ID No. 11, Seq ID No. 13, Seq ID No. 15, Seq ID No. 17, Seq ID No. 19, Seq ID No. 21, Seq ID No. 23, Seq ID No. 25 and Seq ID No. 27.

In this connection, the nucleic acid sequence group that is preferred according to the invention is group A).

A "wild type" of a cell preferably means, in connection with the present invention, the starting strain from which the cell according to the invention was derived by manipulation of the elements (for example the genes comprising the aforesaid nucleic acid sequences coding for a corresponding enzyme or the promoters contained in the corresponding gene, which are linked functionally with the aforesaid nucleic acid sequences), which influence the activities of the enzymes encoded by the stated nucleic acid Seq ID No. If for example the activity of the enzyme encoded by Seq ID No. 1 in the strain ATCC 20336 is reduced by interruption of the corresponding gene, then the strain ATCC 20336 that is unchanged and was used for the corresponding manipulation is to be regarded as the "wild type".

The term "gene" means, in connection with the present invention, not only the encoding DNA region or that transcribed to mRNA, the "structural gene", but in addition promoter, possible intron, enhancer and other regulatory sequence, and terminator, regions.

The term "activity of an enzyme" always means, in connection with the invention, the enzymatic activity that catalyses the reactions of 12-hydroxydodecanoic acid to

1,12-dodecane diacid by the entire cell. This activity is preferably determined by the following method:

Starting from a single colony, a 100-ml Erlenmeyer flask with 10 ml of YM medium (0.3% yeast extract, 0.3% malt extract, 0.5% peptone and 1.0% (w/w) glucose) is cultivated at 30°C and 90 rpm for 24 h. Then, starting from this culture, 10 ml is inoculated into a 1-litre Erlenmeyer flask with 100 ml of production medium (for 1 litre: 25 g glucose, 7.6 g NH_4Cl , 1.5 g Na_2SO_4 , 300 ml of a 1 mM potassium phosphate buffer (pH 7.0), 20 mg $\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}$, 20 mg $\text{MnSO}_4 \times 4 \text{ H}_2\text{O}$, 20 mg nicotinic acid, 20 mg pyridoxine, 8 mg thiamine and 6 mg pantothenate). It is cultivated for 24 h at 30°C.

After 24 h, 12-hydroxydodecanoic acid is added to the cell suspension, so that the concentration is not greater than 0.5 g/l. Glucose or glycerol is also added as co-substrate, so that the concentration of the co-substrate does not drop below 0.2 g/l. After 0h, 0.5 h, 1h, and then hourly up to a cultivation time of 24 h, samples (1 ml) are taken for measurement of 12-hydroxydodecanoic acid, 12-oxo-dodecanoic acid and 1,12-dodecane diacid and the corresponding methyl esters, and for checking the cell count. After each measurement, the pH is kept between 5.0 and 6.5 with 6N NaOH or 4N H_2SO_4 . During cultivation, cell growth is verified by checking the "colony forming units" (CFU). The decrease of 12-hydroxydodecanoic acid and the production of 1,12-dodecane diacid or the corresponding methyl esters are verified by LC-MS. For this, 500 μl of culture broth is adjusted to pH 1 and then extracted with the same volume of diethyl ether or ethyl acetate and analysed by LC-MS.

The measuring system consists of an HP1100 HPLC (Agilent Technologies, Waldbronn, Germany) with degasser, autosampler and column furnace, coupled to a mass-

selective quadrupole detector MSD (Agilent Technologies, Waldbronn, Germany). Chromatographic separation is achieved on a reversed phase e.g. 125x2 mm Luna C18(2) column (Phenomenex, Aschaffenburg, Germany) at 40°C. Gradient elution is performed at a flow of 0.3 ml/min (A: 0.02% formic acid in water and B: 0.02% formic acid in acetonitrile). Alternatively, the organic extracts are analysed by GC-FID (Perkin Elmer, Rodgau-Jügesheim, Germany). Chromatographic separation is performed on a methylpolysiloxane (5% phenyl) phase e.g. Elite 5, 30 m, 0.25 mm ID, 0.25 µm FD (Perkin Elmer, Rodgau-Jügesheim, Germany). Before measurement, a methylation reagent e.g. trimethylsulphonium hydroxide "TMSH" (Macherey-Nagel GmbH & Co. KG, Düren, Germany) is added to free acids and on injection they are converted to the corresponding methyl esters.

By calculating the measured concentration of 1,12-dodecane diacid and the cell number at the time of sampling, it is possible to determine the specific production rate of 1,12-dodecane diacid from 12-hydroxydodecanoic acid and therefore the "activity of an enzyme" in a cell as defined above. The formulation "reduced activity compared with its wild type" means an activity relative to the wild-type activity preferably reduced by at least 50%, especially preferably by at least 90%, more preferably by at least 99.9%, even more preferably by at least 99.99% and most preferably by at least 99.999%.

The decrease in activity of the cell according to the invention compared with its wild type is determined by the method described above for determining activity using cell numbers/concentrations as identical as possible, the cells having been grown under the same conditions, for example medium, gassing, agitation.

"Nucleotide identity" relative to the stated sequences can be determined using known methods. Generally, special computer programs are used with algorithms taking special requirements into account. Preferred methods for determining identity first produce the greatest agreement between the sequences to be compared. Computer programs for determining identity comprise, but are not restricted to, the GCG software package, including

- GAP (Deveroy, J. et al., Nucleic Acid Research 12 (1984), page 387, Genetics Computer Group University of Wisconsin, Medicine (Wi), and

- BLASTP, BLASTN and FASTA (Altschul, S. et al., Journal of Molecular Biology 215 (1990), pages 403-410. The BLAST program can be obtained from the National Center for Biotechnology Information (NCBI) and from other sources (BLAST Manual, Altschul S. et al., NCBI NLM NIH Bethesda ND 22894; Altschul S. et al., above).

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

Preferred parameters for the determination of "nucleotide identity" are, when using the BLASTN program (Altschul, S. et al., Journal of Molecular Biology 215 (1990), pages 403-410):

Expect Threshold:	10
Word size:	28
Match score:	1
Mismatch score:	-2
Gap costs:	linear

The above parameters are the default parameters in nucleotide sequence comparison.

The GAP program is also suitable for use with the above parameters.

An identity of 80% according to the above algorithm means, in connection with the present invention, 80% identity. The same applies to higher identities.

The term "that are encoded by the intron-free nucleic acid sequences" makes clear that in a sequence comparison with the sequences given here, the nucleic acid sequences to be compared must be purified of any introns beforehand.

All stated percentages (%) are percentages by weight unless stated otherwise.

Methods of lowering enzymatic activities in microorganisms are known by a person skilled in the art.

In particular, techniques in molecular biology can be used for this. A person skilled in the art can find instructions on modification and decrease of protein expression and the associated decrease in enzyme activity especially for *Candida tropicalis*, in particular for interrupting specified genes, in WO91/006660; WO03/100013; Picataggio et al. Mol Cell Biol. 1991 Sep; 11(9):4333-9; Rohrer et al. Appl Microbiol Biotechnol. 1992 Feb; 36(5):650-4; Picataggio et al. Biotechnology (N Y). 1992 Aug; 10(8):894-8; Ueda et al. Biochim Biophys Acta. 2003 Mar 17; 1631(2):160-8; Ko et al. Appl Environ Microbiol. 2006 Jun; 72(6):4207-13; Hara et al. Arch Microbiol. 2001 Nov; 176(5):364-9; Kanayama et al. J Bacteriol. 1998 Feb; 180(3): 690-8.

Cells preferred according to the invention are characterized in that the decrease in enzymatic activity is achieved by modification of at least one gene comprising one of the sequences selected from the

previously stated nucleic acid sequence groups A) and B), the modification being selected from the group comprising, preferably consisting of, insertion of foreign DNA into the gene, deletion at least of parts of the gene, point mutations in the gene sequence and subjecting the gene to the influence of RNA interference or exchange of parts of the gene with foreign DNA, in particular of the promoter region.

Foreign DNA means, in this context, any DNA sequence that is "foreign" to the gene (and not to the organism), i.e. even *Candida tropicalis* endogenous DNA sequences can, in this context, function as "foreign DNA".

In this context, it is in particular preferable for the gene to be interrupted by insertion of a selection marker gene, therefore the foreign DNA is a selection marker gene, the insertion preferably having been effected by homologous recombination into the gene locus.

In this context, it may be advantageous if the selection marker gene is expanded with further functionalities, which in their turn make subsequent removal from the gene possible, this can be achieved for example with a Cre/loxP system, with *Flippase Recognition Targets* (FRT) or by homologous recombination.

Cells preferred according to the invention are characterized in that they are blocked in their β -oxidation at least partially, preferably completely, as this prevents outflow of substrate and therefore higher titres become possible.

Examples of *Candida tropicalis* cells partially blocked in their β -oxidation are described in EP0499622 as strains H41, H41B, H51, H45, H43, H53, H534, H534B and H435, from which a *Candida tropicalis* cell preferred according to the invention is derived.

Other *Candida tropicalis* cells blocked for β -oxidation are described for example in WO03/100013.

In this context, cells are preferred for which the β -oxidation is caused by an induced malfunction of at least one of the genes POX2, POX4 or POX5.

Therefore, in this context, cells are preferred that are characterized in that a *Candida tropicalis* cell preferred according to the invention is derived from strains selected from the group comprising ATCC 20962 and the *Candida tropicalis* HDC100 described in US2004/0014198.

The use of the cells according to the invention for the production of ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters also contributes to solution of the task facing the invention.

In particular, the use of the cells according to the invention for the production of ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters with a chain length of the carboxylic acid from 6 to 24, preferably 8 to 18 and especially preferably 10 to 16 carbon atoms, which are preferably linear, saturated and unsubstituted, and a chain length of the alcohol component of the ester from 1 to 4, in particular 1 or 2 carbon atoms, is advantageous. In this context, it is preferable for the ω -hydroxycarboxylic acids to be 12-hydroxydodecanoic acid and for the ω -hydroxycarboxylic acid ester to be 12-hydroxydodecanoic acid methyl ester.

A preferred use is characterized according to the invention in that preferred cells according to the invention as described above are used.

Another contribution to solving the task facing the invention is made by a method of production of the *C. tropicalis* cell according to the invention described above comprising the steps:

I) Preparation of a *C. tropicalis* cell, preferably a cell that is blocked in its β -oxidation at least partially, preferably completely

II) Modification of at least one gene comprising one of the intron-free nucleic acid sequences selected from the previously stated nucleic acid sequence groups A) and B) by insertion of foreign DNA, in particular of DNA coding for a selection marker gene, into the gene, deletion of at least parts of the gene, point mutations in the gene sequence and subjecting the gene to the influence of RNA interference or exchange of parts of the gene with foreign DNA, in particular of the promoter region.

Another contribution to solving the task facing the invention is made by a method of production of ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters, in particular of ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters with a chain length of the carboxylic acid from 6 to 24, preferably 8 to 18 and especially preferably 10 to 16 carbon atoms, which are preferably linear, saturated and unsubstituted, and a chain length of the alcohol component of the ester from 1 to 4, in particular of 1 or 2 carbon atoms, in particular of 12-hydroxydodecanoic acid or 12-hydroxydodecanoic acid methyl ester comprising the steps

A) contacting a previously described cell according to the invention with a medium comprising a carboxylic acid or a carboxylic acid ester, in particular a carboxylic acid or a carboxylic acid ester with a chain length of the carboxylic acid from 6 to 24, preferably 8 to 18 and

especially preferably 10 to 16 carbon atoms, which are preferably linear, saturated and unsubstituted, and a chain length of the alcohol component of the ester from 1 to 4 carbon atoms, in particular dodecanoic acid or dodecanoic acid methyl ester,

B) cultivating the cell under conditions that enable the cell to form the corresponding ω -hydroxycarboxylic acid or ω -hydroxycarboxylic acid esters from the carboxylic acid or the carboxylic acid ester and

C) optionally isolating the ω -hydroxycarboxylic acid or ω -hydroxycarboxylic acid esters that formed.

Preferred methods according to the invention use cells stated above as being preferred according to the invention.

Therefore, for example a method of production of 12-hydroxydodecanoic acid or 12-hydroxydodecanoic acid methyl ester comprising the steps

a) contacting a *Candida tropicalis* cell of the strain ATTC 20336 at least partially blocked in its β -oxidation, which has, compared with its wild type, a reduced activity of at least one of the enzymes, which are encoded by the intron-free nucleic acid sequences selected from the previously stated nucleic acid sequence groups A) and B), the decrease in enzymatic activity being achieved by modification of a gene comprising one of the nucleic acid sequences selected from the previously stated nucleic acid sequence groups A) and B), wherein the modification consists of insertion of a selection marker gene into the gene, with a medium comprising dodecanoic acid or dodecanoic acid methyl ester,

b) cultivating the cell under conditions that enable the cell to form the corresponding ω -hydroxycarboxylic acid or ω -hydroxycarboxylic acid esters from the carboxylic acid or the carboxylic acid ester and

c) optionally isolating the ω -hydroxycarboxylic acid or ω -hydroxycarboxylic acid esters that formed

is quite especially preferred.

Suitable cultivation conditions for *Candida tropicalis* are known by a person skilled in the art. In particular, suitable conditions for step b) are those that are known by a person skilled in the art from bioconversion methods of production of dicarboxylic acids with *Candida tropicalis*.

These cultivation conditions are described for example in WO00/017380 and WO00/015828.

Methods for isolating the ω -hydroxycarboxylic acid or ω -hydroxycarboxylic acid esters that formed are known by a person skilled in the art. These are standard methods for isolating long-chain carboxylic acids from aqueous solution, for example distillation or extraction, and can for example also be found in WO2009/077461.

It is advantageous to use the ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters obtained by the method according to the invention for the production of polymers, in particular polyesters. Moreover, lactones can also be produced from the ω -hydroxy carboxylic acids, and can then for example be used in their turn for the production of polyesters.

Another advantageous use is to convert the ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters

to ω -aminocarboxylic acids or ω -aminocarboxylic acid esters, in order to obtain polyamides as polymers. The ω -aminocarboxylic acids or ω -aminocarboxylic acid esters can also be converted first to the corresponding lactams, which can then in their turn be converted using anionic, or also acid catalysis to a polyamide.

It is quite especially advantageous, in a first reaction step, to convert the ω -hydroxycarboxylic acids or corresponding esters into the ω -oxo-carboxylic acids or the corresponding esters and then to carry out amination of the oxo-group, e.g. in the course of reductive amination.

In this context, the use of 12-hydroxy dodecanoic acid or 12-hydroxydodecanoic acid methyl ester for the production of polymers, in particular of polyamide 12, is especially preferred.

Claims

1. A *Candida tropicalis* cell, characterized in that the cell has, compared with its wild type, a reduced activity of at least one of the enzymes that are encoded by the intron-free nucleic acid sequences selected from the two groups A) and B) consisting of
 - A) Seq ID No. 57, Seq ID No. 61, and Seq ID No. 63
 - B) a sequence that is identical to at least 80% to one of the Seq ID No. 57, Seq ID No. 61, and Seq ID No. 63where the activity of the enzymes is to be understood as meaning the enzymatic activity which catalyses the conversions of 12-hydroxydodecanoic acid into 1,12-dodecandioic acid by the entire cell, furthermore characterized in that the decrease in enzymatic activity is achieved by modification of a gene comprising one of the abovementioned nucleic acid sequences, the modification being selected from the group comprising insertion of foreign DNA into the gene, deletion of at least parts of the gene, point mutations in the gene sequence, subjecting the gene to the influence of RNA interference and exchange of parts of the gene with foreign DNA, in particular of the promoter region.
2. A *Candida tropicalis* cell according to claim 1, characterized in that the foreign DNA is a selection marker gene.
3. A *Candida tropicalis* cell according to at least one of the preceding claims, characterized in that the cell is blocked at least partially in its β -oxidation.
4. A *Candida tropicalis* cell according to at least one of the preceding claims, characterized in that the *Candida tropicalis* cell is derived from strains selected from the group comprising *Candida tropicalis* H41, *Candida tropicalis* H41B, *Candida tropicalis* H51, *Candida tropicalis* H45, *Candida tropicalis* H43, *Candida tropicalis* H53, *Candida tropicalis* H534, *Candida tropicalis* 534B, *Candida tropicalis* H435, *Candida tropicalis*

ATCC20962 and *Candida tropicalis* HDC100, in particular consisting of *Candida tropicalis* ATCC20962 and *Candida tropicalis* HDC100.

5. Use of the cells according to at least one of the preceding claims for the production of ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters.
6. A method of production of a *C. tropicalis* cell according to at least one of claims 1 to 4 comprising the steps:
 - I) preparation of a *C. tropicalis* cell and
 - II) modification of at least one gene comprising one of the sequences selected from the nucleic acid sequence groups A) and B) stated in claim 1 by insertion of foreign DNA, in particular of DNA coding for a selection marker gene, into the gene, deletion at least of parts of the gene, point mutations in the gene sequence, subjecting the gene to the influence of RNA interference and exchange of parts of the gene with foreign DNA, in particular of the promoter region.
7. A method of production of ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters, in particular of ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters with a chain length of the carboxylic acid from 6 to 24 carbon atoms and a chain length of the alcohol component of the ester from 1 to 4 carbon atoms, in particular of 12-hydroxydodecanoic acid or 12-hydroxydodecanoic acid methyl ester comprising the steps
 - a) contacting the cell according to at least one of claims 1 to 4 with a medium comprising a carboxylic acid or a carboxylic acid ester,
 - b) cultivating the cell under conditions that enable the cell to form the corresponding ω -hydroxycarboxylic acid or ω -hydroxycarboxylic acid esters from the carboxylic acid or the carboxylic acid ester and

- c) optionally isolating the ω -hydroxycarboxylic acid or ω -hydroxycarboxylic acid esters that formed.
8. Method of production of a polymer, comprising the method of production of ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters according to Claim 7 and the method step
- use of the ω -hydroxycarboxylic acids or of the ω -hydroxycarboxylic acid esters for the production of a polymer.
9. Method of production of ω -aminocarboxylic acids or ω -aminocarboxylic acid esters, comprising the method steps
- a) contacting a *Candida tropicalis* cell according to at least one of Claims 1 to 4 with a medium comprising a carboxylic acid or a carboxylic acid ester,
 - b) cultivating the cell under conditions that enable the cell to form the corresponding ω -hydroxycarboxylic acid or ω -hydroxycarboxylic acid ester from the carboxylic acid or the carboxylic acid ester,
 - c) optionally isolating the ω -hydroxycarboxylic acid or ω -hydroxycarboxylic acid ester formed and
- a reaction step for converting the ω -hydroxycarboxylic acids or corresponding esters into the ω -oxocarboxylic acids or the corresponding ester and subsequent amination of the oxo group.
10. Method according to Claim 9, characterized in that the ω -hydroxycarboxylic acid or the ω -hydroxycarboxylic acid ester has a carboxylic acid chain length of 6 to 24 carbon atoms and an alcohol component chain length of the ester of 1 to 4 carbon atoms.
11. Method according to Claim 9 or 10, characterized in that the ω -hydroxycarboxylic acid or the ω -hydroxycarboxylic acid ester is 12-hydroxydodecanoic acid or 12-hydroxydodecanoic acid methyl ester.