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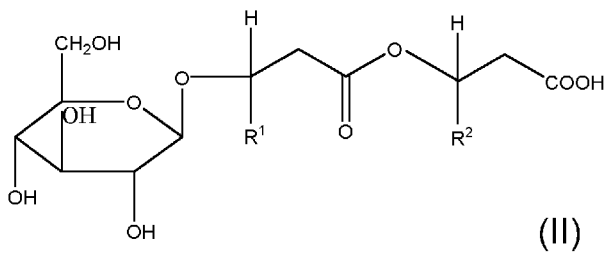

(II)

(57) **Abstract:** The present invention relates to at least one cell for producing at least one lipid with general formula (II) from at least one carbon substrate, wherein R^1 and R^2 independently of one another comprises identical or different organic radicals each with 5 to 13 carbon atoms, wherein the cell is a non-pathogenic cell that is genetically modified to increase the heterologous expression relative to the wild type cell of: - an enzyme (E_2) capable of converting 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP or 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) and NDP-glucose into β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate.

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LIPID PRODUCTION

FIELD OF THE INVENTION

The present invention relates to a recombinant cell and a biotechnological method for producing lipids. In particular, the cell is a non-pathogenic cell genetically modified to produce at least one rubiwettin.

BACKGROUND OF THE INVENTION

Today, most of the available surfactants such as sodium laureth sulfate (SLES), betaine and the like are produced chemically at an industrial scale. These chemically produced surfactants have all the disadvantages that usually come with the use of a chemical production process such as the formation of harmful byproducts. For example, in the SLES production process, at least one harmful byproduct 1,4-dioxane is produced. In order to reduce the amount of toxic products generated and in view of consumers' increasing demand for environmentally friendly products, there is a general trend towards production and use of biosurfactants. Besides producing less poisonous byproducts during the manufacture process, biosurfactants also have useful properties like high structural diversity, beneficial surfactant properties, low environmental toxicity, antibiotic or bioactive properties and complete biological degradability. There is thus a general impetus towards producing and using biosurfactants instead of chemical surfactants.

Rubiwettins are at least one example of such a biosurfactant. Rubiwettins represent an economically interesting class of surfactants because they may potentially replace chemically produced surfactants.

Rubiwettins are exolipids composed of one β-D-glucose molecule linked to a 3-hydroxy fatty acid dimer with chain length C_{14} and C_{10} as lipid main components. They have surface-active properties. Rubiwettins are currently being synthesized by a wildtype *Serratia rubidaea* isolate which is a human- and animal pathogen. The fact that this production organism is able to cause diseases considerably reduces the customer acceptance for these conventionally produced rubiwettins. Further, higher safety requirements are also needed during the production process of rubiwettins and this increases the costs owing to increased capital expenditure and possibly additional production steps.

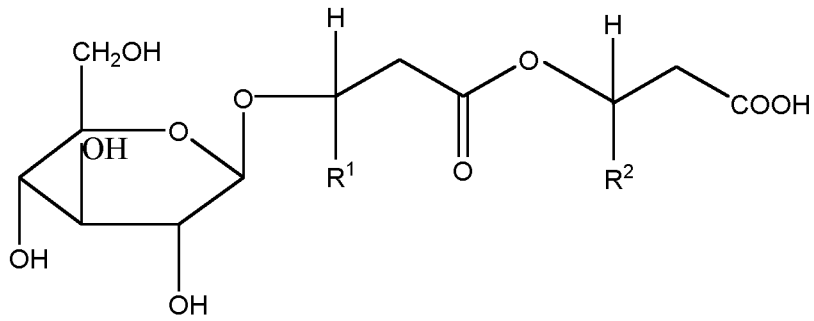
The current methods available for production of biosurfactants such as rubiwettins involve the use of these pathogenic organisms. The yield of production can be optimized by varying pH, oxygen supply, media composition, feeding strategies, nitrogen supply, temperature, choice of substrate and the like. However, if rubiwettins are to be employed on a large scale as surfactants they will have to compete with the currently employed surfactants. The latter

are bulk chemicals, which can be produced at a very low cost. Therefore, rubiwettins must also be produced at costs as low as possible, without health risks for the customer and with defined properties as far as possible. This is not possible by merely optimizing the performance parameters via process optimization.

Accordingly, there is a need in the art for a cheaper and more efficient method of producing biosurfactants, for example rubiwettins with high product yields.

DESCRIPTION OF THE INVENTION

The present invention attempts to solve the problems above by providing a biotechnological means of producing biosurfactants such as lipids, in particular rubiwettins from a carbon source using a non-pathogenic cell. In particular, the non-pathogenic cell may be genetically modified to increase the expression of at least one enzyme (E_2) that is capable of converting an enzyme (E_2) capable of converting 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and/or 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) in combination with NDP-glucose into β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate. The genetically modified cell may then be used to convert a suitable carbon source to a lipid with general formula II below:



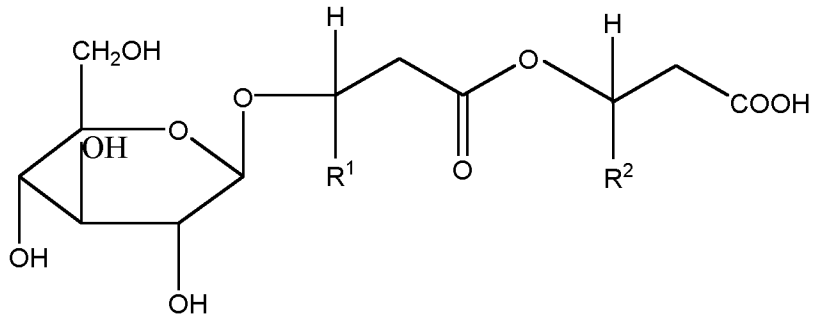
General Formula II

wherein R^1 and R^2 independently of one another is an identical or different alkyl group with 5 to 13 carbon atoms. In particular, the alkyl group may be saturated or unsaturated. More in particular, the alkyl group of R^1 and/or R^2 may be a monounsaturated alkyl radical. Even more in particular, R^1 and/or R^2 may be selected from the group consisting of pentenyl, heptenyl, nonenyl, undecenyl, tridecenyl and $(CH_2)_n$-CH_3 with n=4-12.

This lipid of General Formula II may also be known as a glycolipid and more particularly a rubiwettin RG1. The genetically modified cell according to any aspect of the present invention has the advantage of being non-pathogenic and simple to culture. This enables the cell to be safer for production and also keeps the costs lower as no special safety requirements are needed in the lab during production and use of the rubiwettin. The cells according to any aspect of the present invention has the further advantage of being able to

use a variety of carbon substrates to produce the lipids according to any aspect of the present invention. For examples simple carbons such as glucose may be used as a carbon substrate. Also, the lipids formed according to any aspect of the present invention have defined and flexible properties. It is another advantage according to any aspect of the present invention that rubiwettins can be produced using a non-pathogenic cell. A further advantage is that rubiwettins can be produced with higher space-time yield, higher carbon yields, product concentration, product homogeneity (fatty acid species) than with cells without enhancement of these activities.

According to one aspect of the present invention, there is provided a microbial cell for producing at least one lipid with general formula II from at least one carbon substrate,



General Formula II

wherein R^1 and R^2 independently of one another is an identical or different alkyl group with 5 to 13 carbon atoms, and
wherein the cell is a non-pathogenic cell that is genetically modified to increase the heterologous expression relative to the wild type cell of:

- an enzyme (E_2) capable of converting 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and/or 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) into β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate.

In particular, glucose may be added for either or both of the conversions to take place. Glucose may thus be added for the conversion of 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP into β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate. In another example, glucose may be added for conversion of HAA into β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate. In another example, both HAA and 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP may be present at the same time for β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate production.

The enzyme E_2 may be capable of converting 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP into β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate. In one example,

the enzyme E_2 may be capable of converting HAA into β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate. In yet another example, the enzyme E_2 may be capable of converting 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and HAA into β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate. In all these examples, NDP-glucose may be present particularly to include the glucose moiety in General Formula II. The enzyme E_2 may be a glycosyltransferase (EC 2.4). In particular, the enzyme E_2 comprises SEQ ID NO: 4 or variant thereof. The term "variant", as used herein, comprises amino acid or nucleic acid sequences, respectively, that are at least 60, 65, 70, 75, 80, 85, 90, 92, 94, 95, 96, 97, 98 or 99 % identical to the reference amino acid or nucleic acid sequence, wherein preferably amino acids other than those essential for the function, for example the catalytic activity of a protein, or the fold or structure of a molecule are deleted, substituted or replaced by insertions or essential amino acids are replaced in a conservative manner to the effect that the biological activity of the reference sequence or a molecule derived therefrom is preserved. The state of the art comprises algorithms that may be used to align two given nucleic acid or amino acid sequences and to calculate the degree of identity, see Arthur Lesk (2008), Thompson *et al.*, 1994, and Katoh *et al.*, 2005. The term "variant" is used synonymously and interchangeably with the term "homologue". Such variants may be prepared by introducing deletions, insertions or substitutions in amino acid or nucleic acid sequences as well as fusions comprising such macromolecules or variants thereof. In one example, the term "variant", with regard to amino acid sequence, comprises, in addition to the above sequence identity, amino acid sequences that comprise one or more conservative amino acid changes with respect to the respective reference or wild type sequence or comprises nucleic acid sequences encoding amino acid sequences that comprise one or more conservative amino acid changes. In one example, the term "variant" of an amino acid sequence or nucleic acid sequence comprises, in addition to the above degree of sequence identity, any active portion and/or fragment of the amino acid sequence or nucleic acid sequence, respectively, or any nucleic acid sequence encoding an active portion and/or fragment of an amino acid sequence. The term "active portion", as used herein, refers to an amino acid sequence or a nucleic acid sequence, which is less than the full length amino acid sequence or codes for less than the full length amino acid sequence, respectively, wherein the amino acid sequence or the amino acid sequence encoded, respectively retains at least some of its essential biological activity. For example an active portion and/or fragment of a protease may be capable of hydrolysing peptide bonds in polypeptides. The phrase "retains at least some of its essential biological activity", as used herein, means that the amino acid sequence in question has a biological activity exceeding and distinct from the background activity and the kinetic parameters characterising said activity, more specifically k_{cat} and K_M, are preferably within 3, 2, or 1 order of magnitude of the values displayed by the reference molecule with respect to a specific substrate. Similarly, the term "variant" of a nucleic acid comprises nucleic acids the complementary strand of which hybridises,

preferably under stringent conditions, to the reference or wild type nucleic acid. In one example, the variant of SEQ ID: 4 may have 60% sequence identity to SEQ ID NO:4.

In one example, the enzyme E_2 may have polypeptide sequence SEQ ID NO: 4 or a polypeptide sequence in which up to 25%, preferably up to 20%, particularly preferably up to 15% in particular up to 10, 9, 8, 7, 6, 5, 4, 3, 2, 1% of the amino acid radicals are modified compared to the reference sequence SEQ ID NO: 4 by deletion, insertion, substitution or a combination thereof and that still has at least 10%, preferably 50%, particularly preferably 80%, in particular more than 90% of the enzymatic activity of the enzyme having the reference sequence SEQ ID NO: 4, wherein enzymatic activity for an enzyme E_2 is understood as meaning the ability preferably to convert 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP or 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) into β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate. NDP-glucose may be present in this conversion.

The lipid of general formula II may also be called a rubiwettin. The length of the R^1 and R^2 group can be of varying lengths. In particular, R^1 and R^2 may be independently selected from the group consisting of saturated and unsaturated alkyls. More in particular, R^1 and/or R^2 may be a saturated or unsaturated alkyl group with 5 to 13 carbon atoms. Even more in particular, R^1 and R^2 may be a saturated or monounsaturated alkyl group with 5 to 13 carbon atoms. The R^1 and R^2 alkyl groups may comprise 5 to 13 carbon atoms, 7 to 13 carbon atoms, 9 to 13 carbon atoms, 5 to 11 carbon atoms, 5 to 9 carbon atoms and the like. In the examples where R^1 and/or R^2 alkyl group is a saturated alkyl group, the alkyl group may comprising 5, 6, 7, 8, 9, 10, 11, 12 or 13 carbon atoms. In the examples where R^1 and/or R^2 alkyl group is an unsaturated alkyl group, the alkyl group may be a monounsaturated alkyl comprising 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, or 13:1 carbon atoms. The cell according to any aspect of the present invention may be able to produce a mixture of rubiwettins with varying R^1 and R^2 groups. In one example, the lipid of general formula II produced according to any aspect of the present invention may be a rubiwettin RG1 (CAS-Nr. 129039-46-9). The rubiwettin RG1 may also be called a glycolipid named β-D-Glucopyranosyl-3-(3'-hydroxytetradecanoyloxy)decanoate or β-Glucopyranosyl-3-(3'-hydroxytetradecanoyloxy)decanoate.

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

- an enzyme (E_1) capable of converting 3-hydroxyalkanoyl-CoA/ACP into 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and further to 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA).

The enzyme E_1 may be a 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) synthase. In one example, the enzyme E_1 comprises SEQ ID NO: 2 or variant thereof. In another example, the enzyme E_1 comprises a sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 and variants thereof, wherein the variant comprises 60% sequence identity to SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12 and SEQ ID NO: 14 respectively.

In one example, the enzyme E_1 may have polypeptide sequence SEQ ID NO: 6 or a polypeptide sequence in which up to 25%, preferably up to 20%, particularly preferably up to 15% in particular up to 10, 9, 8, 7, 6, 5, 4, 3, 2, 1% of the amino acid radicals are modified compared to the reference sequence SEQ ID NO: 6 by deletion, insertion, substitution or a combination thereof and that still has at least 10%, preferably 50%, particularly preferably 80%, in particular more than 90% of the enzymatic activity of the enzyme having the reference sequence SEQ ID NO: 6, wherein enzymatic activity for an enzyme E_1 is understood as meaning the ability preferably to convert 3-hydroxyalkanoyl-CoA/ACP into 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and further to HAA.

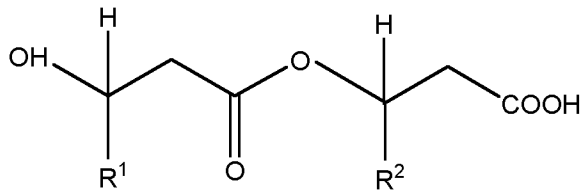
In another example, the enzyme E_1 may have polypeptide sequence SEQ ID NO: 8 or a polypeptide sequence in which up to 25%, preferably up to 20%, particularly preferably up to 15% in particular up to 10, 9, 8, 7, 6, 5, 4, 3, 2, 1% of the amino acid radicals are modified compared to the reference sequence SEQ ID NO: 8 by deletion, insertion, substitution or a combination thereof and that still has at least 10%, preferably 50%, particularly preferably 80%, in particular more than 90% of the enzymatic activity of the enzyme having the reference sequence SEQ ID NO: 8, wherein enzymatic activity for an enzyme E_1 is understood as meaning the ability preferably to convert 3-hydroxyalkanoyl-CoA/ACP into 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and further to 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA).

In a further example, the enzyme E_1 may have polypeptide sequence SEQ ID NO: 10 or a polypeptide sequence in which up to 25%, preferably up to 20%, particularly preferably up to 15% in particular up to 10, 9, 8, 7, 6, 5, 4, 3, 2, 1% of the amino acid radicals are modified compared to the reference sequence SEQ ID NO: 10 by deletion, insertion, substitution or a combination thereof and that still has at least 10%, preferably 50%, particularly preferably 80%, in particular more than 90% of the enzymatic activity of the enzyme having the reference sequence SEQ ID NO: 10, wherein enzymatic activity for an enzyme E_1 is understood as meaning the ability preferably to convert 3-hydroxyalkanoyl-CoA/ACP into 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and further to 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA).

In yet another example, the enzyme E_1 may have polypeptide sequence SEQ ID NO: 12 or a polypeptide sequence in which up to 25%, preferably up to 20%, particularly preferably up to 15% in particular up to 10, 9, 8, 7, 6, 5, 4, 3, 2, 1% of the amino acid radicals are modified compared to the reference sequence SEQ ID NO: 12 by deletion, insertion, substitution or a combination thereof and that still has at least 10%, preferably 50%, particularly preferably 80%, in particular more than 90% of the enzymatic activity of the enzyme having the reference sequence SEQ ID NO: 12, wherein enzymatic activity for an enzyme E_1 is understood as meaning the ability preferably to convert 3-hydroxyalkanoyl-CoA/ACP into 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and further to 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA).

In one other example, the enzyme E_1 may have polypeptide sequence SEQ ID NO: 14 or a polypeptide sequence in which up to 25%, preferably up to 20%, particularly preferably up to 15% in particular up to 10, 9, 8, 7, 6, 5, 4, 3, 2, 1% of the amino acid radicals are modified compared to the reference sequence SEQ ID NO: 14 by deletion, insertion, substitution or a combination thereof and that still has at least 10%, preferably 50%, particularly preferably 80%, in particular more than 90% of the enzymatic activity of the enzyme having the reference sequence SEQ ID NO: 14, wherein enzymatic activity for an enzyme E_1 is understood as meaning the ability preferably to convert 3-hydroxyalkanoyl-CoA/ACP into 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and further to 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA).

The cell according to any aspect of the present invention may produce a further lipid with general formula I from a carbon substrate,



General Formula I

wherein R^1 and R^2 independently of one another is an identical or different alkyl group with 5 to 13 carbon atoms. In particular, the alkyl group may be saturated or unsaturated. More in particular, the alkyl group of R^1 and/or R^2 may be a monounsaturated alkyl radical. Even more in particular, R^1 and/or R^2 may be selected from the group consisting of pentenyl, heptenyl, nonenyl, undecenyl, tridecenyl and $(CH_2)_n$-CH_3 with n=4-12.

The lipid of general formula I may have R^1 and R^2 groups of varying lengths. In particular, R^1 and R^2 may be independently selected from the group consisting of saturated and unsaturated alkyls. More in particular, R^1 and/or R^2 may be a saturated or unsaturated alkyl

group with 5 to 13 carbon atoms. Even more in particular, R^1 and R^2 may be a saturated or monounsaturated alkyl group with 5 to 13 carbon atoms. The R^1 and R^2 alkyl groups may comprise 5 to 13 carbon atoms, 7 to 13 carbon atoms, 9 to 13 carbon atoms, 5 to 11 carbon atoms, 5 to 9 carbon atoms and the like. In the examples where R^1 and/or R^2 alkyl group is an unsaturated alkyl group, the alkyl group may be a monounsaturated alkyl comprising 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, or 13:1 carbon atoms. In the examples where R^1 and/or R^2 alkyl group is a saturated alkyl group, the alkyl group may comprising 5, 6, 7, 8, 9, 10, 11, 12 or 13 carbon atoms. The cell according to any aspect of the present invention may be able to produce a mixture of lipids with varying R^1 and R^2 groups. In particular, the lipid with formula I may also be called a rubiwettin R1 (CAS-Nr. 129039-45-8). In particular, the lipid is a mixture of 3-(3'-hydroxytetradecanoyloxy)tetradecanoate, 3-(3'-hydroxydecanoyloxy)decanoate, 3-(3'-hydroxyhexadecenoyloxy)hexadecenoate, 3-(3'-hydroxytetradecanoyloxy)decanoate, 3-(3'-hydroxyhexadecenoyloxy)decanoate, 3-(3'-hydroxyhexadecenoyloxy)tetradecanoate and minor molecular isomers.

Surprisingly, it could be shown that recombinant cells according to any aspect of the present invention with increased expression of E_2 and/or E_1 are able to produce increased amounts of lipids with the formulas II and/or I compared to the wildtype of the cell. The cells according to any aspect of the present invention may thus allow for high selective production of rubiwettins RG1 with reduced production of undesirable intermediates like dimers of β-hydroxy fatty acids.

The phrase "increased heterologous expression of an enzyme", as used herein is to be understood as increased intracellular activity. Basically, an increase in enzymatic activity can be achieved by increasing the copy number of the gene sequence or gene sequences that code for the enzyme, using a strong promoter or employing a gene or allele that codes for a corresponding enzyme with increased activity and optionally by combining these measures. Genetically modified cells used in the method according to the invention are for example produced by transformation, transduction, conjugation or a combination of these methods with a vector that contains the desired gene, an allele of this gene or parts thereof and a vector that makes expression of the gene possible. Heterologous expression is in particular achieved by integration of the gene or of the alleles in the chromosome of the cell or an extrachromosomally replicating vector. In particular, an increase in an activity of an enzyme relative to the wild type cell may be a 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100% more than the wild type cell.

A skilled person would be able to use any method known in the art to genetically modify a cell. Whether or not a nucleic acid molecule, polypeptide, more specifically an enzyme used according to any aspect of the present invention, is recombinant or not has not necessarily

implications for the level of its expression. However, in one example one or more recombinant nucleic acid molecules, polypeptides or enzymes used according to any aspect of the present invention may be overexpressed. The term “overexpressed”, as used herein, means that the respective polypeptide encoded or expressed is expressed at a level higher or at higher activity than would normally be found in the cell under identical conditions in the absence of genetic modifications carried out to increase the expression, for example in the respective wild type cell. The person skilled in the art is familiar with numerous ways to bring about overexpression. For example, the nucleic acid molecule to be overexpressed or encoding the polypeptide or enzyme to be overexpressed may be placed under the control of a strong inducible promoter such as the lac promoter. The state of the art describes standard plasmids that may be used for this purpose, for example the pET system of vectors exemplified by pET-3a (commercially available from Novagen). Whether or not a nucleic acid or polypeptide is overexpressed may be determined by way of quantitative PCR reaction in the case of a nucleic acid molecule, SDS polyacrylamide electrophoreses, Western blotting or comparative activity assays in the case of a polypeptide. Genetic modifications may be directed to transcriptional, translational, and/or post-translational modifications that result in a change of enzyme activity and/or selectivity under selected and/or identified culture conditions. Thus, in various examples of the present invention, to function more efficiently, a microorganism may comprise one or more gene deletions. Gene deletions may be accomplished by mutational gene deletion approaches, and/or starting with a mutant strain having reduced or no expression of one or more of these enzymes, and/or other methods known to those skilled in the art.

DE-A-100 31 999 gives a general survey of the possibilities for increasing the enzyme activity in cells as exemplified by pyruvate carboxylase, which is inserted hereby as a reference and whose disclosure content with respect to the possibilities for increasing the enzyme activity in cells forms a part of the disclosure of the present invention.

The expression of the above and all subsequently mentioned enzymes or genes is detectable with the aid of 1- and 2-dimensional protein gel separation and subsequent optical identification of the protein concentration in the gel using appropriate analytical software. If the increase in an enzyme activity is based exclusively on an increase in the expression of the corresponding gene, the quantification of the increase in the enzyme activity can be determined in a simple manner by a comparison of the 1- or 2-dimensional protein separations between wild-type and genetically modified cell. A customary method for the preparation of the protein gels in the case of coryneforme bacteria and for the identification of the proteins is the procedure described by Hermann *et al.* (Electrophoresis, 22: 1712.23 (2001)). The protein concentration can likewise be analyzed by Western Blot hybridization using an antibody specific for the protein to be detected (Sambrook *et al.*,

Molecular Cloning: a laboratory manual, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. USA, 1989) and subsequent optical analysis using appropriate software for the concentration determination (Lohaus and Meyer (1989) Biospektrum, 5: 32-39; Lottspeich (1999) Angewandte Chemie 111: 2630-2647). The activity of DNA-binding proteins can be measured by means of DNA band shift assays (also called gel retardation) (Wilson *et al.* (2001) Journal of Bacteriology, 183: 2151-2155). The action of DNA-binding proteins on the expression of other genes can be detected by various well-described methods of the reporter gene assay (Sambrook *et al.*, Molecular Cloning: a laboratory manual, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. USA, 1989). The intracellular enzymatic activities can be determined according to various described methods (Donahue *et al.* (2000) Journal of Bacteriology 182 (19): 5624-5627; Ray *et al.* (2000) Journal of Bacteriology 182 (8): 2277-2284; Freedberg *et al.* (1973) Journal of Bacteriology 115 (3): 816-823). If in the following embodiments no practical methods are indicated for the determination of the activity of a certain enzyme, the determination of the increase in the enzyme activity and also the determination of the decrease of an enzyme activity preferably take place by means of the methods described in Hermann *et al.*, Electophoresis, 22: 1712-23 (2001), Lohaus *et al.*, Biospektrum 5 32-39 (1998), Lottspeich, Angewandte Chemie 111: 2630-2647 (1999) and Wilson *et al.*, Journal of Bacteriology 183: 2151-2155 (2001).

If the increase in the enzyme activity is accomplished by mutation of the endogenous gene, such mutations can be randomly produced either by conventional methods, such as, for example, by UV irradiation or by mutagenic chemicals, or selectively by means of genetic engineering methods such as deletion(s), insertion(s) and/or nucleotide exchange(s). Modified cells are obtained by these mutations. Particularly preferred mutants of enzymes are in particular also those enzymes that are no longer feedback-, product- or substrate-inhibitable or are so to a reduced degree at least in comparison to the wild-type enzyme.

If the increase in the enzyme activity is accomplished by increase in the synthesis of an enzyme, the copy number of the corresponding genes is increased or the promoter and regulation region or the ribosome binding site, which is situated upstream of the structural gene, is mutated. Expression cassettes, which are incorporated upstream of the structural gene, act in the same manner. It is additionally possible, by means of inducible promoters, to increase the expression at any desired point in time. In addition, however, also "enhancers" can be assigned to the enzyme gene as regulatory sequences, which likewise bring about increased gene expression by means of an improved interaction between RNA polymerase and DNA. As a result of measures for the prolongation of the lifetime of the mRNA, the expression is likewise improved. Furthermore, by prevention of the degradation of the enzyme protein the enzyme activity is likewise increased. The genes or gene constructs are

present here either in plasmids having a different copy number or are integrated and amplified in the chromosome. Alternatively, an overexpression of the genes concerned can furthermore be achieved by modification of the media composition and culture management. The person skilled in the art finds directions for this, inter alia, in Martin *et al.* (Bio/Technology 5, 137-146 (1987)), in Guerrero *et al.* (Genes 138, 35-41 (1994)), Tsuchiya and Morinaga (Bio/Technology 6, 428-430 (1988)), in Eikmanns *et al.* (Genes 102, 93-98 (1991)), in EP-A-0 472 869, in US 4,601,893, in Schwarzer and Pühler (Bio/Technology 9, 84-87 (1991)), in Reinscheid *et al.* (Applied and Environmental Microbiology 60, 126-132 (1994)), in LaBarre *et al.* (Journal of Bacteriology 175, 1001-1007 (1993)), in WO-A-96/15246, in Malumbres *et al.* (Genes 134, 15-24 (1993)), in JP-A-10-229891, in Jensen and Hammer (Biotechnology and Bioengineering 58, 191-195 (1998)) and in known textbooks of genetics and molecular biology. The measures described above likewise lead, like the mutations, to genetically modified cells.

Episomal plasmids, for example, are employed for increasing the expression of the respective genes. Suitable plasmids or vectors are in principle all embodiments available for this purpose to the person skilled in the art. Such plasmids and vectors can be taken, for example, from the brochures of the companies Novagen, Promega, New England Biolabs, Clontech or Gibco BRL. Further preferred plasmids and vectors can be found in: Glover, D. M. (1985) DNA cloning: a practical approach, Vol. I-III, IRL Press Ltd. , Oxford; Rodriguez, R.L. and Denhardt, D. T (eds) (1988) Vectors : a survey of molecular cloning vectors and their uses, 179-204, Butterworth, Stoneham; Goeddel, D. V. (1990) Systems for heterologous gene expression, Methods Enzymol. 185, 3-7; Sambrook, J.; Fritsch, E. F. and Maniatis, T. (1989), Molecular cloning: a laboratory manual, 2nd ed., Cold Spring Harbor Laboratory Press, New York.

The plasmid vector, which contains the gene to be amplified, is then converted to the desired strain by conjugation or transformation. The method of conjugation is described, for example, in Schäfer *et al.*, Applied and Environmental Microbiology 60: 756-759 (1994). Methods for transformation are described, for example, in Thierbach *et al.*, Applied Microbiology and Biotechnology 29: 356-362 (1988), Dunican and Shivnan, Bio/Technology 7: 1067-1070 (1989) and Tauch *et al.*, FEMS Microbiology Let-ters 123: 343-347 (1994). After homologous recombination by means of a “cross-over” event, the resulting strain contains at least two copies of the gene concerned.

According to any aspect of the present invention, the cell may be genetically modified so that in a defined time interval, within 2 hours, in particular within 8 hours or 24 hours, it forms at least twice, especially at least 10 times, at least 100 times, at least 1000 times or at least 10000 times more lipids of the general Formula I or II than the wild-type cell. The increase in product formation can be determined for example by cultivating the cell according to any

aspect of the present invention and the wild-type cell each separately under the same conditions (same cell density, same nutrient medium, same culture conditions) for a specified time interval in a suitable nutrient medium and then determining the amount of target product (lipid with general formula II or I) in the nutrient medium.

Changes of amino acid residues of a given polypeptide sequence, which lead to no significant changes in the properties and function of the given polypeptide, are known to the person skilled in the art. Thus, for example, “conserved amino acids” can be mutually exchanged; examples of such suitable amino acid substitutions are: Ala for Ser; Arg for Lys; Asn for Gln or His; Asp for Glu; Cys for Ser; Gln for Asn; Glu for Asp; Gly for Pro; His for Asn or Gln; Ile for Leu or Val; Leu for Met or Val; Lys for Arg or Gln or Glu; Met for Leu or Ile; Phe for Met or Leu or Tyr; Ser for Thr; Thr for Ser; Trp for Tyr; Tyr for Trp or Phe; Val for Ile or Leu. It is likewise known that changes, particularly at the N- or C-terminus of a polypeptide, in the form of, for example, amino acid insertions or deletions often exert no significant influence on the function of the polypeptide.

The activity of an enzyme can be determined by disrupting cells which contain this activity in a manner known to the person skilled in the art, for example with the aid of a ball mill, a French press or of an ultrasonic disintegrator and subsequently separating off cells, cell debris and disruption aids, such as, for example, glass beads, by centrifugation for 10 minutes at 13,000 rpm and 4°C. Using the resulting cell-free crude extract, enzyme assays with subsequent LC-ESI-MS detection of the products can then be carried out. Alternatively, the enzyme can be enriched in the manner known to the person skilled in the art by chromatographic methods (such as nickel-nitrilotriacetic acid affinity chromatography, streptavidin affinity chromatography, gel filtration chromatography or ion-exchange chromatography) or else purified to homogeneity.

In one example the method used to determine the activity of enzyme E_2 involves first disrupting cells which contain this activity (i.e. the cells according to any aspect of the present invention) in a manner known to the person skilled in the art, for example with the aid of a ball mill, a French press or an ultrasonic disintegrator and subsequently separating of cells, cell debris and disruption aids, such as, for example, glass beads, by centrifugation for 10 min at 16,100 g at 4° C. Using the resulting cell-free crude extract, enzyme assays with subsequent LC-ESI-MS detection of the products can be carried out. As an alternative, the enzyme can be enriched in the manner known to the person skilled in the art by chromatography methods (such as nickel/nitrilotriacetic acid affinity chromatography, streptavidin affinity chromatography, gel filtration chromatography or ion-exchange chromatography) or else purified to homogeneity. This sample may then be used to measure the activity of enzyme E_2. In particular, the activity of enzyme E_2 may be determined using a

standard assay that may consists of 185 µl of 10 mM Tris-HCl (pH 7.5), 10 µl mM NDP-glucose and 50 µl of protein crude extract (about 1 mg of total protein) or purified protein in solution (5 µg of purified protein). The reaction may be started by the addition of 10 µl mM ethanolic solution of 3-hydroxytetradecanoyl-3-hydroxydecanoic acid or 3-hydroxyhexadecanoyl-3-hydroxydecanoic acid and incubated for 1 h at 30° C with shaking (600 rpm). Subsequently, the reaction may be treated with 1 ml of acetone. Undissolved constituents, may be sedimented by centrifugation (16,100 g, 5 min RT) and the sample may be analyzed by means of LC-ESI-MS. The identification of the products may then take place by analysis of the corresponding mass traces and the MS2 spectra. This method may be used to measure the activity of E_2.

In another example, the method used to determine the activity of enzyme E_1 involves first disrupting cells which contain this activity (i.e. the cells according to any aspect of the present invention) in a manner known to the person skilled in the art and subsequently separating of cells, cell debris and disruption aids, such as, for example, glass beads, by centrifugation for 10 min at 16,100 g at 4° C. Using the resulting cell-free crude extract, enzyme assays with subsequent LC-ESI-MS detection of the products can be carried out. As an alternative, the enzyme can be enriched in the manner known to the person skilled in the art by chromatography methods (such as nickel/nitrilotriacetic acid affinity chromatography, streptavidin affinity chromatography, gel filtration chromatography or ion-exchange chromatography) or else purified to homogeneity. This sample may then be used to measure the activity of enzyme E_1. In particular, the activity of enzyme E_1 may be determined using a standard assay which may contain 100 µM *E. coli* ACP, 1 mM β-mercaptoethanol, 200 µM malonyl-coenzyme A, 40 µM octanoyl-coenzyme A and 40 µM dodecanoyl-coenzyme A or 40 µM octanoyl-coenzyme A and 40 mM tetradecanoyl-coenzyme A, 100 µM NADPH, 2 µg of *E. coli* FabD, 2 µg of *Mycobacterium tuberculosis* FabH, 1 µg of *E. coli* FabG, 0.1 M sodium phosphate buffer (pH 7.0), and 5 µg of enzyme E_1 in a final volume of 120 µl. ACP, β-mercaptoethanol and sodium phosphate buffer may be preincubated for 30 min at 37° C to reduce the ACP completely. The reaction may be started by addition of enzyme E_1. The reactions may be stopped using 2 ml of water, which has been acidified with HCl to pH 2.0, and subsequently extracted twice with 2 ml of chloroform/methanol (2:1 (v:v)). Phase separation may take place by centrifugation (16,100 g, 5 min, RT). The lower organic phase may be removed, evaporated completely in the vacuum centrifuge and the sediment may be taken up in 50 µl of methanol. Undissolved constituents, may be sedimented by centrifugation (16,100 g, 5 min RT) and the sample may be analyzed by means of LC-ESI-MS. The identification of the products may take place by analysis of the corresponding mass traces and the MS2 spectra.

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

The cell used according to any aspect of the present invention may also be a non-pathogenic cell. A non-pathogenic cell refers to a cell that does not cause disease, harm or death to another organism. The cells according to any aspect of the present invention may any non-pathogenic prokaryote or eukaryote. These can be mammalian cells (such as, for example, cells from man), plant cells or microorganisms such as yeasts, fungi or bacteria, wherein microorganisms in particular bacteria and yeasts are preferred.

Suitable bacteria, yeasts or fungi are in particular those bacteria, yeasts or fungi that are deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures) GmbH (DSMZ), Brunswick, Germany, as bacterial, yeast or fungal strains. Bacteria suitable according to the invention belong to the genera that are listed under:

- http://www.dsmz.de/species/bacteria.htm,

yeasts suitable according to the invention belong to those genera that are listed under:

- http://www.dsmz.de/species/yeasts.htm

and fungi suitable according to the invention are those that are listed under:

- http://www.dsmz.de/species/fungi.htm.

In particular, the cells may be selected from the genera *Aspergillus*, *Corynebacterium*, *Brevibacterium*, *Bacillus*, *Acinetobacter*, *Alcaligenes*, *Lactobacillus*, *Paracoccus*, *Lactococcus*, *Candida*, *Pichia*, *Hansenula*, *Kluyveromyces*, *Saccharomyces*, *Escherichia*, *Zymomonas*, *Yarrowia*, *Methylobacterium*, *Ralstonia*, *Pseudomonas*, *Rhodospirillum*, *Rhodobacter*, *Burkholderia*, *Clostridium and Cupriavidus*. More in particular, the cells may be selected from the group consisting of *Aspergillus nidulans*, *Aspergillus niger*, *Alcaligenes latus*, *Bacillus megaterium*, *Bacillus subtilis*, *Brevibacterium flavum*, *Brevibacterium lactofermentum*, *Burkholderia andropogonis*, *B. brasilensis*, *B. caledonica*, *B. caribensis*, *B. caryophylli*, *B. fungorum*, *B. gladioli*, *B. glathei*, *B. glumae*, *B. graminis*, *B. hospita*, *B. kururiensis*, *B. phenazinium*, *B. phymatum*, *B. phytofirmans*, *B. plantarii*, *B. sacchari*, *B. singaporensis*, *B. sordidicola*, *B. terricola*, *B. tropica*, *B. tuberum*, *B. ubonensis*, *B. unamae*,

B. xenovorans, *B. anthina*, *B. pyrrocinia*, *B. thailandensis*, *Candida blankii*, *Candida rugosa*, *Corynebacterium glutamicum*, *Corynebacterium efficiens*, *Escherichia coli*, *Hansenula polymorpha*, *Kluveromyces lactis*, *Methylobacterium extorquens*, *Paracoccus versutus*, *Pseudomonas argentinensis*, *P. borbori*, *P. citronellolis*, *P. flavescens*, *P. mendocina*, *P. nitroreducens*, *P. oleovorans*, *P. pseudoalcaligenes*, *P. resinovorans*, *P. straminea*, *P. aurantiaca*, *P. aureofaciens*, *P. chlororaphis*, *P. fragi*, *P. lundensis*, *P. taetrolens*, *P. antarctica*, *P. azotoformans*, *'P. blatchfordae'*, *P. brassicacearum*, *P. brenneri*, *P. cedrina*, *P. corrugata*, *P. fluorescens*, *P. gessardii*, *P. libanensis*, *P. mandelii*, *P. marginalis*, *P. mediterranea*, *P. meridiana*, *P. migulae*, *P. mucidolens*, *P. orientalis*, *P. panacis*, *P. proteolytica*, *P. rhodesiae*, *P. synxantha*, *P. thivervalensis*, *P. tolaasii*, *P. veronii*, *P. denitrificans*, *P. pertucinogena*, *P. cremoricolorata*, *P. fulva*, *P. monteilii*, *P. mosselii*, *P. parafulva*, *P. putida*, *P. balearica*, *P. stutzeri*, *P. amygdali*, *P. avellanae*, *P. caricapapayae*, *P. cichorii*, *P. coronafaciens*, *P. ficuserectae*, *'P. helianthi'*, *P. meliae*, *P. savastanoi*, *P. syringae*, *P. tomato*, *P. viridiflava*, *P. abietaniphila*, *P. acidophila*, *P. agarici*, *P. alcaliphila*, *P. alkanolytica*, *P. amyloderamosa*, *P. asplenii*, *P. azotifigens*, *P. cannabina*, *P. coenobios*, *P. congelans*, *P. costantinii*, *P. cruciviae*, *P. delhiensis*, *P. excibis*, *P. extremorientalis*, *P. frederiksbergensis*, *P. fuscovaginae*, *P. gelidicola*, *P. grimontii*, *P. indica*, *P. jessenii*, *P. jinjuensis*, *P. kilonensis*, *P. knackmussii*, *P. koreensis*, *P. lini*, *P. lutea*, *P. moraviensis*, *P. otitidis*, *P. pachastrellae*, *P. palleroniana*, *P. papaveris*, *P. peli*, *P. perolens*, *P. poae*, *P. pohangensis*, *P. psychrophila*, *P. psychrotolerans*, *P. rathonis*, *P. reptilivora*, *P. resiniphila*, *P. rhizosphaerae*, *P. rubescens*, *P. salomonii*, *P. segitis*, *P. septica*, *P. simiae*, *P. suis*, *P. thermotolerans*, *P. aeruginosa*, *P. tremae*, *P. trivialis*, *P. turbinellae*, *P. tuticorinensis*, *P. umsongensis*, *P. vancouverensis*, *P. vranovensis*, *P. xanthomarina*, *Ralstonia eutropha*, *Rhodospirillum rubrum*, *Rhodobacter sphaeroides*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica* and *Zymomonas mobile*. More in particular, the cell may be a bacterial cell selected from the group consisting of *Acinetobacter sp.*, *Bacillus sp.*, *Brevibacterium sp.*, *Burkholderia sp.*, *Chlorella sp.*, *Clostridium sp.*, *Corynebacterium sp.*, *Cyanobakterien*, *Escherichia sp.*, *Pseudomonas sp.*, *Klebsiella sp.*, *Salmonella sp.*, *Rhizobium sp.*, *Saccharomyces sp.*, *Pichia sp.*, and *Nostoc sp.*. Even more in particular, the cell may be selected from the group consisting of *Bacillus subtilis, Burkholderia thailandensis, Corynebacterium glutamicum, E. coli, Klebsiella oxytoca, Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas stutzeri, Rhizobium meliloti, Saccharomyces cerevisiae* and *Pichia pastoris.*

In one example, the cell according to any aspect of the present invention may be a cell that is genetically modified to increase the expression of

- enzyme E_2 comprises SEQ ID NO: 4 or variant thereof, and
- enzyme E_1 comprises SEQ ID NO: 2 or variant thereof.

The cell according to any aspect of the present invention may be a non-pathogenic cell that is genetically modified to increase the heterologous expression relative to the wild type cell of:

- an enzyme (E_2) capable of converting 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and/or 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) to β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate, wherein E_2 is a glycosyltransferase (EC 2.4); and
- an enzyme (E_1) capable of converting 3-hydroxyalkanoyl-CoA/ACP into 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and further to 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA), wherein E_1 is a 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) synthase.

The cell according to a further aspect of the present invention may be a non-pathogenic cell that is genetically modified to increase the heterologous expression relative to the wild type cell of:

- an enzyme (E_2) capable of converting 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP or HAA to β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate, wherein E_2 is a glycosyltransferase (EC 2.4) that comprises SEQ ID NO:4; and
- an enzyme (E_1) capable of converting 3-hydroxyalkanoyl-CoA/ACP into 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and further to 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA), wherein E_1 is a 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) synthase that comprises SEQ ID NO:2.

The cell according to any aspect of the present invention may be a non-pathogenic cell that is genetically modified to increase the heterologous expression relative to the wild type cell of:

- an enzyme (E_2) capable of converting 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP or 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) to β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate, wherein E_2 is a glycosyltransferase (EC 2.4); and
- an enzyme (E_1) capable of converting 3-hydroxyalkanoyl-CoA/ACP into 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and further to HAA, wherein E_1 is a 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) synthase that comprises SEQ ID NO:6.

The cell according to one other aspect of the present invention may be a non-pathogenic cell that is genetically modified to increase the heterologous expression relative to the wild type cell of:

- an enzyme (E_2) capable of converting 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP or HAA to β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate, wherein E_2 is a glycosyltransferase (EC 2.4); and
- an enzyme (E_1) capable of converting 3-hydroxyalkanoyl-CoA/ACP into 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and further to 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA), wherein E_1 is a 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) synthase that comprises SEQ ID NO:8.

The cell according to yet another aspect of the present invention may be a non-pathogenic cell that is genetically modified to increase the heterologous expression relative to the wild type cell of:

- an enzyme (E_2) capable of converting 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP or HAA to β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate, wherein E_2 is a glycosyltransferase (EC 2.4); and
- an enzyme (E_1) capable of converting 3-hydroxyalkanoyl-CoA/ACP into 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and further to 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA), wherein E_1 is a 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) synthase that comprises SEQ ID NO:10.

The cell according to a further aspect of the present invention may be a non-pathogenic cell that is genetically modified to increase the heterologous expression relative to the wild type cell of:

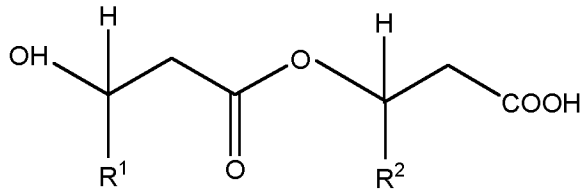
- an enzyme (E_2) capable of converting 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP or HAA to β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate, wherein E_2 is a glycosyltransferase (EC 2.4) that comprises SEQ ID NO:4; and
- an enzyme (E_1) capable of converting 3-hydroxyalkanoyl-CoA/ACP into 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and further to 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA), wherein E_1 is a 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) synthase that comprises SEQ ID NO:12.

The cell according to a further aspect of the present invention may be a non-pathogenic cell that is genetically modified to increase the heterologous expression relative to the wild type cell of:

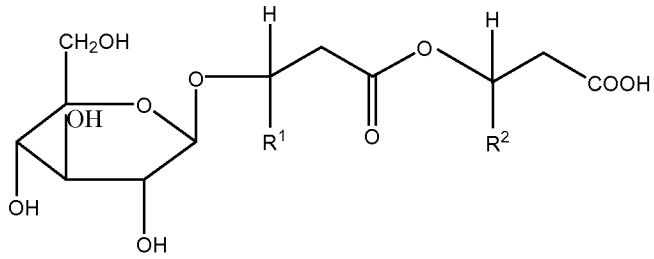
- an enzyme (E_2) capable of converting 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP or HAA to β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate, wherein E_2 is a glycosyltransferase (EC 2.4) that comprises SEQ ID NO:4; and
- an enzyme (E_1) capable of converting 3-hydroxyalkanoyl-CoA/ACP into 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and further to 3-(3-

hydroxyalkanoyloxy)alkanoic acid (HAA), wherein E_1 is a 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) synthase that comprises SEQ ID NO:14.

The cells according to any aspect of the present invention may be used to produce a lipid according to General formula I and/or II from a carbon substrate:



General Formula I



General Formula II

wherein R^1 and R^2 independently of one another in General Formula I or II is an identical or different alkyl group with 5 to 13 carbon atoms. In particular, the alkyl group may be saturated or unsaturated. More in particular, the alkyl group of R^1 and/or R^2 may be a monounsaturated alkyl radical. Even more in particular, R^1 and/or R^2 may be selected from the group consisting of pentenyl, heptenyl, nonenyl, undecenyl, tridecenyl and $(CH_2)_n$-CH_3 with n=4-12.

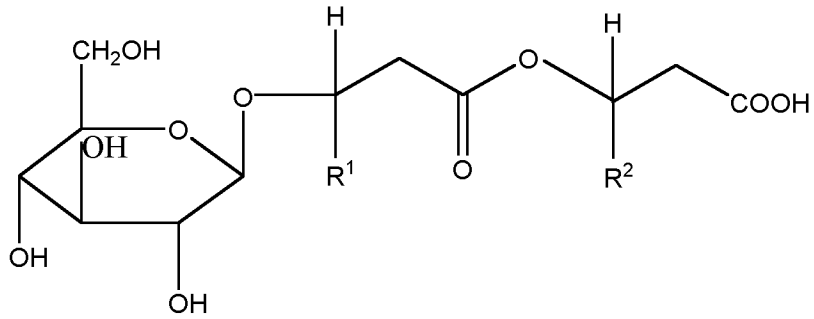
The lipids formed may be combination of lipids with general formula I and II with varying R group that may be produced during a single reaction.

The genetically modified cells according to the invention can be brought into contact with the nutrient medium continuously or discontinuously in the batch process (batch culture) or in the fed-batch process (feed process) or repeated fed-batch process (repetitive feed process) for the purpose of the production of the abovementioned products and thus cultured. A semi-continuous process is also conceivable, as is described in GB-A-1009370. A summary of known culturing methods are described in the textbook of Chmiel ("Bioprozesstechnik 1. Einführung in die Bioverfahrenstechnik" [Bioprocess Technology 1. Introduction to the Bioprocess Technique] (Gustav Fischer Verlag, Stuttgart, 1991)) or in the textbook of Storhas ("Bioreaktoren und periphere Einrichtungen" [Bioreactors and Peripheral Devices], Vieweg Verlag, Brunswick/Wiesbaden, 1994).

The culture medium to be used must satisfy in a suitable manner the demands of the respective strains. Descriptions of culture media of different yeast strains are contained, for example, in “Nonconventional yeast in biotechnology” (Ed. Klaus Wolf, Springer-Verlag Berlin, 1996).

The carbon source used as a substrate according to any aspect of the present invention may be selected from the group consisting of carbohydrates such as, for example, glucose, sucrose, arabinose, xylose, lactose, fructose, maltose, molasses, starch, cellulose and hemicellulose, vegetable and animal oils and fats such as, for example, soybean oil, safflower oil, peanut oil, hempseed oil, jatropha oil, coconut fat, calabash oil, linseed oil, corn oil, poppyseed oil, evening primrose oil, olive oil, palm kernel oil, palm oil, rapeseed oil, sesame oil, sunflower oil, grapeseed oil, walnut oil, wheat germ oil and coconut oil, fatty acids, such as, for example, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, arachidonic acid, behenic acid, oleic acid, linoleic acid, linolenic acid, gamma-linolenic acid and its methyl or ethyl ester as well as fatty acid mixtures, mono-, di- and triglycerides containing the fatty acids just mentioned, alcohols such as, for example, glycerol, ethanol and methanol, hydrocarbons such as methane, ethane, propane or butane carbon-containing gases and gas mixtures, such as CO, CO_2, synthesis or flue gas, amino acids such as L-glutamate or L-valine or organic acids such as, for example, acetic acid. These substances can be used individually or as a mixture. The use of carbohydrates, in particular of monosaccharides, oligosaccharides or polysaccharides, as the carbon source as is described in US 6,136,576 as well as of hydrocarbons, in particular of alkanes, alkenes and alkynes. In particular, the carbon source may be selected from the group consisting of glucose, dextrose, sucrose, polysaccharides, such as cellulose or hemicelluloses, vegetal oils, animal fats, fatty acids, fatty acid esters, carbonaceous gases, alkanes, glycerol, acetate, ethanol and methanol. More in particular, the carbon source may be selected from the group consisting of glucose, sucrose, glycerol, vegetal oils, methane, ethane, and butane. It is a great advantage of the present invention that the cells according to the invention are able to form lipids with general formula I and/or II from the simplest carbon sources such as, for example, glucose, sucrose or glycerol, such that a provision of longer-chain C sources in the medium during the method according to any aspect of the present invention is not necessary.

According to one aspect of the present invention there is provided a method of producing at least one lipid with general formula II:



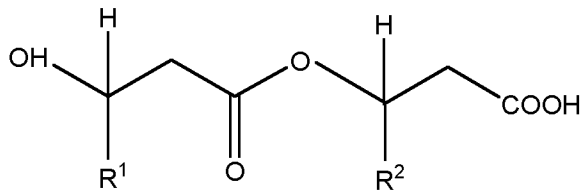
General Formula II

wherein R^1 and R^2 independently of one another is an identical or different alkyl group with 5 to 13 carbon atoms, and

wherein the method comprises a step of contacting at least one cell according to any aspect of the present invention with at least one carbon source.

In particular, the alkyl group of R^1 and/or R^2 may be saturated or unsaturated. More in particular, the alkyl group of R^1 and/or R^2 may be a monounsaturated alkyl radical. Even more in particular, R^1 and/or R^2 may be selected from the group consisting of pentenyl, heptenyl, nonenyl, undecenyl, tridecenyl and $(CH_2)_n$-CH_3 with n=4-12.

The method according to any aspect of the present invention may also be used to produce a further lipid with general formula I from the carbon substrate,



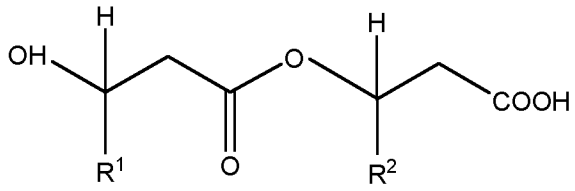
General Formula I

wherein R^1 and R^2 independently of one another is an identical or different alkyl group with 5 to 13 carbon atoms. In particular, the alkyl group of R^1 and/or R^2 may be saturated or unsaturated. More in particular, the alkyl group of R^1 and/or R^2 may be a monounsaturated alkyl radical. Even more in particular, R^1 and/or R^2 may be selected from the group consisting of pentenyl, heptenyl, nonenyl, undecenyl tridecenyl and $(CH_2)_n$-CH_3 with n=4-12.

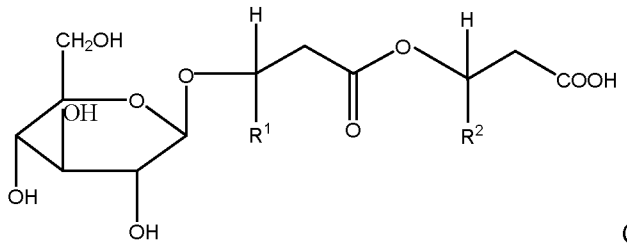
The method according to any aspect of the present invention may be used to produce a mixture of lipids comprising the lipid in general formula I and II. In particular, the lipids of general formula I and II are produced in the ratio of 1:100, 1:90, 1:80, 1:70, 1:60, 1:50, 1:40,

1:30, 1:20, 1:10, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 10:1, 20:1, 30:1, 40:1, 50:1, 60:1, 70:1, 80:1, 90:1 or 100:1. More in particular lipids of formula I and II may have varying lengths of alkyls present simultaneously in the R subgroup.

According to a further aspect of the present invention, there is provided a use of the cell according to any aspect of the present invention for producing at least one lipid with general formula I and/or II:



General Formula I



General Formula II

wherein R^1 and R^2 independently of one another is an identical or different alkyl group with 5 to 13 carbon atoms. In particular, the alkyl group of R^1 and/or R^2 may be saturated or unsaturated. More in particular, the alkyl group of R^1 and/or R^2 may be a monounsaturated alkyl radical. Even more in particular, R^1 and/or R^2 may be selected from the group consisting of pentenyl, heptenyl, nonenyl, undecenyl, tridecenyl and $(CH_2)_n$-CH_3 with n=4-12.

EXAMPLES

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

Example 1

Construction of an expression vector for the Serratia rubidaea genes rbwAB

For the heterologous expression of the genes *rbwA* (SEQ ID NO. 1) as enzyme E_1 and *rbwB* (SEQ ID NO. 3) as enzyme E_2 from *Serratia rubidaea* the plasmid pACYC_rbwAB_Srub was constructed. The synthetic operon consisting of *rbwAB_Srub* (SEQ ID NO: 15) which encode an 3-(3'-

hydroxyalkanoyloxy)alkanoic acids (HAAs) synthase (RbwA, SEQ ID NO: 2) and a glucosyltransferase (RbwB, SEQ ID NO: 4), respectively, was cloned under the control of the rhamnose inducible promoter P_{rha} into the vector pACYCATh-5, which is based on pAYCY184 (New England Biolabs, Frankfurt /Main, Germany). Downstream of the synthetic operon a terminator sequence is located. The genes were amplified from genomic DNA of *S. rubidaea* via PCR. The P_{Rha} promoter cassette (SEQ ID NO: 16) and the terminator sequence (SEQ ID NO: 17) were amplified from *E. coli* K12 genomic DNA. The plasmid pACYCATh-5 carries a p15A origin of replication for *E. coli* and a pVS1 origin of replication for the replication in *P. putida* KT2440. The pVS1 origin comes from the *Pseudomonas* plasmid pVS1 (Itoh Y, et al. *Plasmid* 1984, 11(3), 206-20). *rbwA* and *rbwB* were fused via cross-over PCR to generate an optimized operon. For amplification the Phusion™ High-Fidelity Master Mix from New England Biolabs (Frankfurt/Main, Germany) was used according to manufacturer's manual. In the next step the fusion construct was cloned into the vector pACYCATh-5 using the restriction sites *Apa*I/*Psp*XI. The ligated product was transformed into chemically competent *E. coli* DH5α cells (New England Biolabs, Frankfurt/Main, Germany). Procedure of PCR purification, cloning and transformation were carried out according to manufacturer's manual. The correct insertion of the target genes was checked by restriction analysis and the authenticity of the introduced DNA fragments was verified by DNA sequencing. The resulting plasmid was named pACYC_rbwAB_Srub (SEQ ID NO: 18).

The *P. putida* strain KT2440 was transformed with the plasmid pACYC_rbwAB_Srub by means of electroporation (Iwasaki K, et al., *Biosci. Biotech. Biochem.* 1994. 58(5):851-854)) and plated onto LB-agar plates supplemented with kanamycin (50 µg/mL). Transformants were checked for the presence of the correct plasmid by plasmid preparation and analytic restriction analysis. The resulting strain was named BS-PP-360 (*P. putida* KT2440 pACYC_rbwAB_Srub).

Example 2

Construction of an expression vector for the Serratia rubidaea gene rbwA

For the heterologous expression of the gene *rbwA* (SEQ ID NO: 1) from *S. rubidaea* the plasmid pACYC_rbwA_Srub was constructed. For this approach the plasmid pACYC_rbwAB_Srub (see Example 1) was cut with the restriction enzymes *Nsi*I and *Xho*I to eliminate *rbwB*. To re-ligate the modified vector, the plasmid was treated with T4 DNA polymerase (New England Biolabs, Frankfurt /Main, Germany) in order to remove 3' overhangs and to fill-in of 5' overhangs to form blunt ends. The religated product was transformed into chemically competent *E. coli* DH5α cells (New England Biolabs, Frankfurt/Main, Germany). Procedure of PCR purification, cloning and transformation were carried out according to manufacturer's manual. The correct insertion of the target genes was checked by restriction analysis and the authenticity of the introduced DNA fragments was verified by DNA sequencing. The resulting plasmid was named pACYC_rbwA_Srub (SEQ ID NO: 19).

The *P. putida* strain KT2440 was transformed with the plasmid pACYC_rbwA_Srub by means of electroporation (Iwasaki K, et al. *Biosci. Biotech. Biochem.* 1994. 58(5):851-854) and plated onto LB-agar plates supplemented with kanamycin (50 µg/mL). Transformants were checked for the

presence of the correct plasmid by plasmid preparation and analytic restriction analysis. The resulting strain was named BS-PP-433 (*P. putida* KT2440 pACYC_rbwA_Srub).

Example 3

Construction of an expression vector for the P. aeruginosa gene rhlA and S. rubidaea gene rbwB

For the heterologous expression of the gene *rhlA* (SEQ ID NO: 5) from *P. aeruginosa* and *rbwB* (SEQ ID NO: 3) from *S. rubidaea* the plasmid pACYC_rhlA_Pa rbwB_Srub was constructed. The synthetic operon consisting of *rhlA_Pa* (SEQ ID NO: 20) which encodes a 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAAs) synthase (RhlA, SEQ ID NO: 6) and a glucosyltransferase (RbwB, SEQ ID NO: 4), respectively, was cloned under the control of the rhamnose inducible promoter P_{rha} into the vector pACYCATh-5. Downstream of the synthetic operon a terminator sequence is located. The genes were amplified from genomic DNA of *P. aeruginosa* and *S. rubidaea* respectively via PCR. The P_{Rha} promoter cassette (SEQ ID NO: 16) and the terminator sequence (SEQ ID NO: 17) were amplified from *E. coli* K12 genomic DNA. The vector is based on pACYC184 (New England Biolabs, Frankfurt /Main, Germany) and carries a p15A origin of replication for *E. coli* and a pVS1 origin of replication for the replication in *P. putida* KT2440. The pVS1 origin comes from the *Pseudomonas* plasmid pVS1 (Itoh Y, Watson JM, Haas D, Leisinger T, Plasmid 1984, 11(3), 206-20). *rhlA* and *rbwB* were fused via cross-over PCR to generate an optimized operon. For amplification the Phusion™ High-Fidelity Master Mix from New England Biolabs (Frankfurt/Main, Germany) was used according to manufacturer's manual. In the next step the fusion construct was cloned into the vector pACYCATh-5 using the restriction sites *Apa*I/*Psp*XI. The ligated product was transformed into chemically competent *E. coli* DH5α cells (New England Biolabs, Frankfurt/Main, Germany). Procedure of PCR purification, cloning and transformation were carried out according to manufacturer's manual. The correct insertion of the target genes was checked by restriction analysis and the authenticity of the introduced DNA fragments was verified by DNA sequencing. The resulting plasmid was named pACYC_rhlA_Pa rbwB_Srub (SEQ ID NO: 21).

The *P. putida* strain KT2440 was transformed with the plasmid pACYC_rhlA_Pa rbwB_Srub by means of electroporation (Iwasaki K, et al., *Biosci. Biotech. Biochem.* 1994. 58(5):851-854)) and plated onto LB-agar plates supplemented with kanamycin (50 µg/mL). Transformants were checked for the presence of the correct plasmid by plasmid preparation and analytic restriction analysis. The resulting strain was named BS-PP-368 (*P. putida* KT2440 pACYC_rhlA_Pa rbwB_Srub).

Example 4

Production of lipid R1 with strain BS-PP-433 (P. putida KT2440 pACYC_rbwA_Srub)

For the production of lipid R1, DASGIP® parallel bioreactor system from Eppendorf (Hamburg, Germany) is used. The fermentation is performed using 1 L reactors. pH and pO2 are measured

online for process monitoring. OTR/CTR measurements serve for estimating the metabolic activity and cell fitness, inter alia.

The pH electrodes are calibrated by means of a two-point calibration using standard solutions of pH 4.0 and pH7.0, as specified in DASGIP's technical instructions. The reactors are equipped with the necessary sensors and connections as specified in the technical instructions, and the agitator shaft is fitted. The reactors are then filled with 300 ml water and autoclaved for 20 min at 121°C to ensure sterility. The pO2 electrodes are connected to the measuring amplifiers and polarized overnight (for at least 6 h). Thereafter, the water is removed under a clean bench and replaced by fermentation medium (2.2 g/L $(NH_4)_2SO_4$, 0.02 g/L NaCl, 0.4 g/L $MgSO_4$ x $7H_2O$, 0.04 g/L $CaCl_2$ x $2H_2O$, sterilized separately: 2 g/L KH_2PO_4, 8.51 g/L KH_2PO_4, 20 g/L glucose, 10 mL/L trace elements solution M12 (sterile-filtered: 0.2 g/L $ZnSO_4$ x 7 H_2O, 0.1 g/L $MnCl_2$ x $4H_2O$, 1.5 g/L Na_3-Citrat x 2 H_2O, 0.1 g/L $CuSO_4$ x 5 H_2O, 0.002 g/L $NiCl_2$ x 6 H_2O, 0.003 g/L Na_2MoO_4 x 2 H_2O, 0.03 g/L H_3BO_3, 1 g/L $FeSO_4$ x 7 H_2O). Thereafter, the pO2 electrodes are calibrated to 100 % with a one-point calibration (stirrer: 600 rpm/aeration 10 sl/h air), and the feed, correction agent and induction agent lines are cleaned by "cleaning in place" as specified in the technical instructions. To this end, the tubes are rinsed first with 70 % ethanol, then with 1 M NaOH, then with sterile fully-demineralized water and, finally, filled with the respective media.

Using the BS-PP-433 (*P. putida* strain KT2440 pACYC_rbwA_Srub), 25 ml LB1 medium (10 g/L tryptone, 5 g/L yeast extract, 1 g/L NaCl, pH 7.0) supplemented with kanamycin (50 µg/mL) in a baffeled shake flask are inoculated with 100 µl of a glycerol stock solution and incubated for ~18 h over night at 30 °C and 200 rpm. The first preculture is used to inoculate 50 ml seed medium (autoclaved: 4.4 g/L Na_2HPO_4 * 2 H_2O, 1.5 g/L KH_2PO_4, 1 g/L NH_4Cl, 10 g/L yeast extract, sterilized separately: 20 g/L glucose, 0.2 g/L $MgSO_4$ * 7 H_2O, 0.006 g/L $FeCl_3$, 0.015 g/L $CaCl_2$, 1 ml/L trace elements solution SL6 (sterile-filtered: 0.3 g/L H_3BO_3, 0.2 g/L $CoCl_2$ x 6 H_2O, 0.1 g/L $ZnSO_4$ x 7 H_2O, 0.03 g/L $MnCl_2$ x $4H_2O$, 0.01 g/L $CuCl_2$ x 2 H_2O, 0.03 g/L Na_2MoO_4 x 2 H_2O, 0.02 g/L $NiCl_2$ x 6 H_2O) in a 500 ml baffeled shake flask (starting OD_{600} 0.2). The culture is incubated for ~7 h at 200 rpm and 30°C. In order to inoculate the reactors with an optical density of 0.7, the OD_{600} of the second preculture stage is measured and the amount of culture required for the inoculation is calculated.

The required amount of culture is added with the help of a 30 ml syringe through a septum into the heat-treated and aerated reactor.

The standard program shown in Table 1 is used:

a)

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

b)

N (Rotation)	From	To	XO2 (gas mixture)	from	to	F (gas flow)	from	to
Growth and biotrans-formation	0 %	40 %	Growth and biotrans-formation	0 %	100 %	Growth and biotrans-formation	35 %	100 %
	500 rpm	1500 rpm		21 %	21 %		9 sl/h	72 sL/h

c)

Script	
Trigger fires	31 % DO (1/60h)
Temperature	37 °C
Induction rhamnose	3 h after the feed start
Feed trigger	50 % DO
Feed rate	1.5 [mL/h]

Table1. Standard program used for heated and aerated reactor

The pH is adjusted unilaterally to pH 7.0 with 12.5 % strength ammonia solution. During the growth phase and the biotransformation, the dissolved oxygen (pO2 or DO) in the culture is adjusted to at least 30 % via the stirrer speed and the aeration rate. After the inoculation, the DO dropped from 100 % to these 30 %, where it is maintained permanently for the rest of the fermentation.

The fermentation is carried out as a fed batch. The feed starts with a 2.5 g/L*h glucose feed, composed of 500 g/L glucose, and was triggered via the DO peak which indicates the end of the batch phase. 3 h after the feed start, the expression of lipid R1 production was induced with 0.2 % (w/v) rhamnose. The inducer concentration refers to the volume at the beginning of fermentation.

The production of lipid R1 starts with the induction. At specified time points samples are taken from the fermenter to determine the concentration of lipid R1 produced.

The strain BS-PP-433 produces more 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) than the reference strain with an empty plasmid.

Example 5

Production of rubiwettin RG1 with strain BS-PP-360 (P. putida KT2440 pACYC_rbwAB_Srub)

For the production of rubiwettin RG1 the DASGIP® parallel bioreactor system from Eppendorf (Hamburg, Germany) was used. The fermentation was performed using 1 L reactors. pH and pO2 were measured online for process monitoring. OTR/CTR measurements served for estimating the metabolic activity and cell fitness, inter alia.

The pH electrodes were calibrated by means of a two-point calibration using standard solutions of pH 4.0 and pH7.0, as specified in DASGIP's technical instructions. The reactors were equipped with the necessary sensors and connections as specified in the technical instructions, and the agitator shaft was fitted. The reactors were then filled with 300 ml water and autoclaved for 20 min at 121°C to ensure sterility. The pO2 electrodes were connected to the measuring amplifiers and polarized overnight (for at least 6 h). Thereafter, the water was removed under a clean bench and replaced by fermentation medium (2.2 g/L $(NH_4)_2SO_4$, 0.02 g/L NaCl, 0.4 g/L $MgSO_4$ x $7H_2O$, 0.04 g/L $CaCl_2$ x $2H_2O$, sterilized separately: 2 g/L KH_2PO_4, 8.51 g/L KH_2PO_4, 20 g/L glucose, 10 mL/L trace elements solution M12 (sterile-filtered: 0.2 g/L $ZnSO_4$ x 7 H_2O, 0.1 g/L $MnCl_2$ x $4H_2O$, 1.5 g/L Na_3-Citrat x 2 H_2O, 0.1 g/L $CuSO_4$ x 5 H_2O, 0.002 g/L $NiCl_2$ x 6 H_2O, 0.003 g/L Na_2MoO_4 x 2 H_2O, 0.03 g/L H_3BO_3, 1 g/L $FeSO_4$ x 7 H_2O).thereafter, the pO2 electrodes were calibrated to 100 % with a one-point calibration (stirrer: 600 rpm/aeration 10 sl/h air), and the feed, correction agent and induction agent lines were cleaned by "cleaning in place" as specified in the technical instructions. To this end, the tubes were rinsed first with 70 % ethanol, then with 1 M NaOH, then with sterile fully-demineralized water and, finally, filled with the respective media.

Using the *P. putida* strain BS-PP-360, 25 ml LB1 medium (10 g/L tryptone, 5 g/L yeast extract, 1 g/L NaCl, pH 7.0) supplemented with kanamycin (50 µg/mL) in a baffeled shake flask were inoculated with 100 µl of a glycerol stock solution and incubated for ~18 h over night at 30 °C and 200 rpm. The first preculture was used to inoculate 50 ml seed medium (autoclaved: 4.4 g/L Na_2HPO_4 * 2 H_2O, 1.5 g/L KH_2PO_4, 1 g/L NH_4Cl, 10 g/L yeast extract, sterilized separately: 20 g/L glucose, 0.2 g/L $MgSO_4$ * 7 H_2O, 0.006 g/L $FeCl_3$, 0.015 g/L $CaCl_2$, 1 ml/L trace elements solution SL6 (sterile-filtered: 0.3 g/L H_3BO_3, 0.2 g/L $CoCl_2$ x 6 H_2O, 0.1 g/L $ZnSO_4$ x 7 H_2O, 0.03 g/L $MnCl_2$ x $4H_2O$, 0.01 g/L $CuCl_2$ x 2 H_2O, 0.03 g/L Na_2MoO_4 x 2 H_2O, 0.02 g/L $NiCl_2$ x 6 H_2O) in a 500 ml baffeled shake flask (starting OD_{600} 0.2). The culture were incubated for ~7 h at 200 rpm and 30°C. In order to inoculate the reactors with an optical density of 0.7, the OD_{600} of the second preculture stage was measured and the amount of culture required for the inoculation was calculated.

The required amount of culture was added with the help of a 30 ml syringe through a septum into the heat-treated and aerated reactor. The standard program shown in example 4 of table 1 was used.

The pH was adjusted unilaterally to pH 7.0 with 12.5 % strength ammonia solution. During the growth phase and the biotransformation, the dissolved oxygen (pO_2 or DO) in the culture was adjusted to at least 30 % via the stirrer speed and the aeration rate. After the inoculation, the DO dropped from 100 % to these 30 %, where it was maintained stably for the remainder of the fermentation.

The fermentation was carried out as a fed batch. The feed starts with a 2.5 g/L*h glucose feed, composed of 500 g/L glucose, and was triggered via the DO peak which indicates the end of the batch phase. 3 h after the feed start, the expression of rubiwettin production was induced with 0.2 % (w/v) rhamnose. The inducer concentration referred to the volume at the beginning of fermentation. For both sugars stock solution of 220 g/L was used. The production of rubiwettin RG1 started with the induction. At specified time points samples were taken from the fermenter to determine the concentration of rubiwettins produced. After 65 h fermentation 0.53 g/L rubiwettin RG1 was produced.

Example 6

Production of rubiwettin RG1 with strain BS-PP-368 (P. putida KT2440 pACYC_rhlA_Pa rbwB_Srub)

For the production of rubiwettin RG1 the DASGIP® parallel bioreactor system from Eppendorf (Hamburg, Germany) was used. The fermentation was performed using 1 L reactors. pH and pO2 were measured online for process monitoring. OTR/CTR measurements served for estimating the metabolic activity and cell fitness, inter alia.

The pH electrodes were calibrated by means of a two-point calibration using standard solutions of pH 4.0 and pH7.0, as specified in DASGIP's technical instructions. The reactors were equipped with the necessary sensors and connections as specified in the technical instructions, and the agitator shaft was fitted. The reactors were then filled with 300 ml water and autoclaved for 20 min at 121°C to ensure sterility. The pO2 electrodes were connected to the measuring amplifiers and polarized overnight (for at least 6 h). Thereafter, the water was removed under a clean bench and replaced by fermentation medium (2.2 g/L $(NH_4)_2SO_4$, 0.02 g/L NaCl, 0.4 g/L $MgSO_4 \times 7H_2O$, 0.04 g/L $CaCl_2 \times 2H_2O$, sterilized separately: 2 g/L KH_2PO_4, 8.51 g/L KH_2PO_4, 20 g/L glucose, 10 mL/L trace elements solution M12 (sterile-filtered: 0.2 g/L $ZnSO_4 \times 7\ H_2O$, 0.1 g/L $MnCl_2 \times 4H_2O$, 1.5 g/L Na_3-Citrat $\times 2\ H_2O$, 0.1 g/L $CuSO_4 \times 5\ H_2O$, 0.002 g/L $NiCl_2 \times 6\ H_2O$, 0.003 g/L $Na_2MoO_4 \times 2\ H_2O$, 0.03 g/L H_3BO_3, 1 g/L $FeSO_4 \times 7\ H_2O$). Thereafter, the pO2 electrodes were calibrated to 100 % with a one-point calibration (stirrer: 600 rpm/aeration 10 sl/h air), and the feed, correction agent and induction agent lines were cleaned by "cleaning in place" as specified in the technical instructions.

To this end, the tubes were rinsed first with 70 % ethanol, then with 1 M NaOH, then with sterile fully-demineralized water and, finally, filled with the respective media.

Using the *P. putida* strain BS-PP-368, 25 ml LB1 medium (10 g/L tryptone, 5 g/L yeast extract, 1 g/L NaCl, pH 7.0) supplemented with kanamycin (50 µg/mL) in a baffeled shake flask were inoculated with 100 µl of a glycerol stock solution and incubated for ~18 h over night at 30 °C and 200 rpm. The first preculture was used to inoculate 50 ml seed medium (autoclaved: 4.4 g/L Na_2HPO_4 * 2 H_2O, 1.5 g/L KH_2PO_4, 1 g/L NH_4Cl, 10 g/L yeast extract, sterilized separately: 20 g/L glucose, 0.2 g/L $MgSO_4$ * 7 H_2O, 0.006 g/L $FeCl_3$, 0.015 g/L $CaCl_2$, 1 ml/L trace elements solution SL6 (sterile-filtered: 0.3 g/L H_3BO_3, 0.2 g/L $CoCl_2$ x 6 H_2O, 0.1 g/L $ZnSO_4$ x 7 H_2O, 0.03 g/L $MnCl_2$ x 4H_2O, 0.01 g/L $CuCl_2$ x 2 H_2O, 0.03 g/L Na_2MoO_4 x 2 H_2O, 0.02 g/L $NiCl_2$ x 6 H_2O) in a 500 ml baffeled shake flask (starting OD_{600} 0.2). The culture were incubated for ~7 h at 200 rpm and 30°C. In order to inoculate the reactors with an optical density of 0.7, the OD_{600} of the second preculture stage was measured and the amount of culture required for the inoculation was calculated.

The required amount of culture was added with the help of a 30 ml syringe through a septum into the heat-treated and aerated reactor. The standard program shown in table 1 of example 4 was used for the heated and aerated reactor.

The pH was adjusted unilaterally to pH 7.0 with 12.5 % strength ammonia solution. During the growth phase and the biotransformation, the dissolved oxygen (pO_2 or DO) in the culture was adjusted to at least 30 % via the stirrer speed and the aeration rate. After the inoculation, the DO dropped from 100 % to these 30 %, where it was maintained stably for the remainder of the fermentation.

The fermentation was carried out as a fed batch. The feed starts with a 2.5 g/L*h glucose feed, composed of 500 g/L glucose, and was triggered via the DO peak which indicates the end of the batch phase. 3 h after the feed start, the expression of rubiwettin production was induced with 0.2 % (w/v) rhamnose. The inducer concentration referred to the volume at the beginning of fermentation. For both sugars stock solution of 220 g/L was used. The production of rubiwettin RG1 started with the induction. At specified time points samples were taken from the fermenter to determine the concentration of rubiwettins produced. After 65 h fermentation 11.1 g/L rubiwettin RG1 was produced.

Example 7

HPLC-based quantification of rubiwettins

Quantification of lipids R1 and RG1 was carried out by means of HPLC. Using a displacement pipette (Combitip), 900 µl of 70 % (v/v) n-propanol was introduced into a 2 ml reaction vessel and the reaction vessel was immediately closed for minimization of evaporation. The addition of 100 µl fermentation broth followed. After shaking for 1 min in a Retsch mill at a frequency of 30 Hz, the resulting crude extract mixture was centrifuged for 5 min at 13,000 rpm, and 800 µl of the clear supernatant was transferred into an HPLC vial. Further dilutions of cell broth were carried out in 55 % (v/v) propanol. Samples were stored at -20°C before measurement.

For the detection and quantification of lipids an evaporation light scattering detector (Sedex LT-ELSD Model 85LT) was used. The measurement was carried out by means of Agilent Technologies 1200 Series (Santa Clara, Calif.) and a Zorbax SB-C8 Rapid Resolution column (4,6 x 150 mm, 3,5 µm, Agilent). The injection volume was 5.0 µl and the run time was 20 min. Mobile phase A: aqueous 0.1 % TFA (trifluoracetic acid, solution); mobile phase B: methanol. The column temperature was 40 °C. The ELSD (detector temperature 60 °C) and the DAD (diode array, 210 nm) were used as detectors.

Gradient:

t [min]		Flow [1ml/min]
0.00	70 %	1.00
15.00	100 %	1.00
15.01	70 %	1.00
20.00	70 %	1.00

Table 2. Gradient of mobile phases of A and B over time

The gradient used starts with 70 % B in A to 100 % B within 15 minutes at a flow rate of 1 mL/min followed by 5 minutes of re-equilibration with 70 % B in A (see Table 2). Reference materials were used whose identity and purity were checked by HPLC-MS/MS and NMR.

Example 8

Construction of Agrobacterium tumefaciens *strains for production of rubiwettin R1 and rubiwettin RG1*

In order to show production of rubiwettins with yet another microbial species, *Agrobacterium tumefaciens*, we prepare electrocompetent cells of *Agrobacterium tumefaciens* LBA 4404 and transformed it with plasmids *pACYC_rbwA_Srub* (SEQ ID NO: 19)*, pACYC_rbwAB_Srub* (SEQ ID NO: 18) *and* pACYC_rhlA_Pa rbwB_Srub (SEQ ID NO: 21).

To that end, freshly growing cells (1–2 days old) of *A. tumefaciens* LBA 4404 are spread on LB agar plates (diameter 90 mm, 10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl, and 15 g/L agar, supplemented with 50 µg/mL rifampicin) and incubated overnight (~ 16 h) at 27 °C to produce a bacterial lawn that covers the surface of the plate completely.

Bacterial cells are carefully washed off the plate with 4 mL ice-cold 10% (v/v) sterile glycerol. Cells growing on the surface of the plate are scraped off with an inoculation loop avoiding damages of the agar medium and suspended in the glycerol solution. The bacterial suspension is then transferred into two sterile 2 mL centrifuge tubes.

Suspensions in the two tubes are centrifuged at 14,000 rpm (18,000 g) for 1 min at 4 °C; the supernatant is discarded.

1 mL ice-cold 10% (v/v) sterile glycerol is added to each tube containing the bacterial pellet. The tubes are thoroughly vortexed afterwards to resuspend the cells and this washing step is repeated one more time.

After the two centrifugation steps, the supernatant is removed and discarded again and the bacterial pellets in the two tubes are resuspended in 200 µl ice-cold 10% (v/v) sterile glycerol each and combined in one tube (yielding 400 µl in total).

The tube with the *Agrobacterium* cell suspension is kept on ice until electroporation.

For electroporation 70–80 µl of the ice-cold suspension of electrocompetent bacterial cells is mixed with 1–3 µl plasmid DNA (1–100 ng) in a sterile centrifuge tube.

This mixture is loaded into a chilled electroporation cuvette (gap = 2 mm) and placed into the cuvette holder. The electroporator (Gene Pulser Xcell™ Microbial Electroporation Systems; Bio-Rad) is used with the following parameters: 2.5 kV, 25 µF capacitance, and 400 Ohm resistance.

One mL SOC medium (20 mM glucose, 20 g/L tryptone, 5 g/L yeast extract, 10 mM NaCl, 2.5 mM $MgCl_2$, and 10 mM $MgSO_4$) is added immediately to the electroporation cuvette and the resulting bacterial suspension transferred into a 15 mL centrifuge tube, and the tube is incubated at 27 °C for 1 h with rotating.

After incubation 100 µl from each suspension of electroporated cells is spread onto LB plates supplemented with kanamycin (50 µg/mL). The plates are incubated for 2 days at 27 °C and successfully transformed colonies verified by plasmid isolation and analytical restriction digests.

The following strains are generated:

A. tumefaciens LBA 4404 pACYC_rbwA_Srub for production of rubiwettin R1

A. tumefaciens LBA 4404 pACYC_rbwAB_Srub for production of rubiwettin RG1

A. tumefaciens LBA 4404 pACYC_rhlA_Pa rbwB_Srub for production of rubiwettin RG1

Example 9

Production of rubiwettin R1 with Agrobacterium tumefaciens *LBA 4404 pACYC_rbwA_Srub and rubiwettin RG1 with* Agrobacterium tumefaciens *LBA 4404 pACYC_rbwAB_Srub and* Agrobacterium tumefaciens *LBA 4404 pACYC_rhlA_Pa rbwB_Srub*

For the production of rubiwettins R1 and RG1 the DASGIP® parallel bioreactor system from Eppendorf (Hamburg, Germany) is used. The fermentation is performed using 1 L reactors. pH and pO_2 are measured online for process monitoring. OTR/CTR measurements served for estimating the metabolic activity and cell fitness, inter alia.

The pH probes are calibrated by means of a two-point calibration with measurement solutions of pH 4.0 and pH 7.0 according to technical reference of DASGIP. The reactors are provided according to technical reference with the required sensors and connections and the stirrer shaft is installed. The reactors are then filled with 300 ml of water and autoclaved for 20 min at 121°C in order to ensure sterility. The pO2 probes are polarized overnight (at least 6 h) following connection to the measurement amplifier. The water is then removed under the clean bench and replaced by high-cell-density medium consisting of (NH4)2SO4 1.76 g/l, K2HPO4 19.08 g/l, KH2PO4 12.5 g/l, yeast extracts 6.66 g/l, trisodium citrate dihydrate 11.2 g/l, 17 ml/l of a filter-sterilized 1% strength ammonium iron citrate solution, and 5 ml/l of a filter-sterilized trace element stock solution (consisting of HCl (37%) 36.50 g/l, MnCl2*4H2O 1.91 g/l, ZnSO4*7H2O 1.87 g/l,

ethylenediaminetetraacetic acid dihydrate 0.84 g/l, H3BO3 0.30 g/l, Na2MoO4*2H2O 0.25 g/l, CaCl2*2H2O 4.70 g/l, FeSO4*7H2O 17.80 g/l, CuCl2*2H2O 0.15 g/l) with 15 g/l glucose as carbon source (added by metered addition of 30 ml/l of a sterile feed solution consisting of 500 g/l glucose, 1% (w/v) MgSO4*7H2O and 2.2% (w/v) NH4Cl) with 50 mg/l kanamycin.

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

For production of rubiwettin R1 with *Agrobacterium tumefaciens* LBA 4404 pACYC_rbwA_Srub as well as production of rubiwettin RG1 with *Agrobacterium tumefaciens* LBA 4404 pACYC_rbwAB_Srub and *Agrobacterium tumefaciens* LBA 4404 pACYC_rhlA_Pa rbwB_Srub, the three strains are cultured firstly from a cryoculture in LB medium (25 ml in a 100 ml baffled shake flask) with 50 mg/l kanamycin overnight at 28°C and 200 rpm for about 18 h. Then, 2 ml of this culture is transferred for a second preculture stage into 25 ml of high-cell-density medium consisting of (NH4)2SO4 1.76 g/L, K2HPO4 19.08 g/l, KH2PO4 12.5 g/l, yeast extract 6.66 g/l, trisodium citrate dihydrate 11.2 g/l, 17 ml/l of a filter-sterilized 1% strength ammonium iron citrate solution, and 5 ml/l of a filter-sterilized trace element stock solution (consisting of HCl (37%) 36.50 g/l, MnCl2*4H2O 1.91 g/l, ZnSO4*7H2O 1.87 g/l, ethylenediaminetetraacetic acid dihydrate 0.84 g/l, H3BO3 0.30 g/l. Na2MoO4*2H2O 0.25 g/l, CaCl2*2H2O 4.70 g/l, FeSO4*7H2O 17.80 g/l, CuCl2*2H2O 0.15 g/l) with 15 g/l glucose as carbon source (added by metered addition of 30 ml/l of a sterile feed solution consisting of 500 g/l glucose, 1% (w/v) MgSO4*7H2O and 2.2% (w/v) NH4Cl) with the already described antibiotics in a 100 ml shake flask and incubated at 28°C/200 rpm for a further 6 h.

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

The standard program used is shown in Table 3:

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

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

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

Table 3. The standard program for production of rubiwettins with *Agrobacterium* strains

The pH is regulated to pH 6.8 on one side with 12.5% strength ammonia solution. During cultivation and biotransformation, the dissolved oxygen (pO2 or DO) in the culture is regulated to at least 30% by means of stirrer feed and gassing rate. Following inoculation, the DO drops from 100% to this 30%, where it is kept stable for the remainder of the fermentation. The temperature is kept stable at 28°C.

The fermentation is carried out as fed-batch, where the feed start is triggered as delivery to the feed phase with 1.5 g/l*h glucose feed, consisting of 500 g/l glucose, 1% (w/v) $MgSO_4*7H_2O$ and 2.2% (w/v) NH_4Cl, via the DO peak inducing the end of the batch phase. 3 h after the feed start, rubiwettin production is induced with 0.2% (w/v) rhamnose. The inducer concentration refers to the volume at the beginning of fermentation. A rhamnose stock solution of 220 g/L is used.
Quantification of formation of rubiwettins R1 and RG1 is performed as described in Example 7.
It is shown that *Agrobacterium tumefaciens* LBA 4404 pACYC_rbwA_Srub produces rubiwettin R1.
It is also shown that *Agrobacterium tumefaciens* LBA 4404 pACYC_rbwAB_Srub and *Agrobacterium tumefaciens* LBA 4404 pACYC_rhlA_Pa rbwB_Srub both produce rubiwettins RG1.

Example 10

Construction of E. coli *strains for production of rubiwettin R1 and rubiwettin RG1*

The plasmids *pACYC_rbwA_Srub* (SEQ ID NO: 19)*, pACYC_rbwAB_Srub* (SEQ ID NO: 18) *and* pACYC_rhlA_Pa rbwB_Srub (SEQ ID NO: 21)are transformed via electroporation into *E. coli* W3110 and plated onto LB agar plates with kanamycin (50 µg/ml). Transformants are screened for presence and authenticity of the plasmids by plasmid preparation and restriction digest analysis.
The following strains are generated:

E.coli W3110 pACYC_rbwA_Srub for production of rubiwettin R1

E.coli W3110 pACYC_rbwAB_Srub for production of rubiwettin RG1

E.coli W3110 pACYC_rhlA_Pa rbwB_Srub for production of rubiwettin RG1

Example 11

Production of rubiwettin R1 with E. coli *W3110 pACYC_rbwA_Srub and rubiwettin RG1 with* E. coli *W3110 pACYC_rbwAB_Srub and* E. coli *W3110 pACYC_rhlA_Pa rbwB_Srub*

For the production of rubiwettins R1 and RG1 the DASGIP® parallel bioreactor system from Eppendorf (Hamburg, Germany) is used. The fermentation is performed using 1 L reactors. pH and pO_2 are measured online for process monitoring. OTR/CTR measurements served for estimating the metabolic activity and cell fitness, inter alia.

The pH probes are calibrated by means of a two-point calibration with measurement solutions of pH 4.0 and pH 7.0 according to technical reference of DASGIP. The reactors are provided according to technical reference with the required sensors and connections and the stirrer shaft is installed. The reactors are then filled with 300 ml of water and autoclaved for 20 min at 121°C in order to ensure sterility. The pO2 probes are polarized overnight (at least 6 h) following connection to the measurement amplifier. The water is then removed under the clean bench and replaced by high-cell-density medium consisting of $(NH_4)_2SO_4$ 1.76 g/l, K_2HPO_4 19.08 g/l, KH_2PO_4 12.5 g/l, yeast extracts 6.66 g/l, trisodium citrate dihydrate 11.2 g/l, 17 ml/l of a filter-sterilized 1% strength ammonium iron citrate solution, and 5 ml/l of a filter-sterilized trace element stock solution (consisting of HCl (37%) 36.50 g/l, $MnCl_2*4H_2O$ 1.91 g/l, $ZnSO_4*7H_2O$ 1.87 g/l, ethylenediaminetetraacetic acid dihydrate 0.84 g/l, H_3BO_3 0.30 g/l, Na_2MoO_4*2H2O 0.25 g/l, $CaCl_2*2H_2O$ 4.70 g/l, $FeSO_4*7H_2O$ 17.80 g/l, $CuCl_2*2H_2O$ 0.15 g/l) with 15 g/l glucose as carbon source (added by metered addition of 30 ml/l of a sterile feed solution consisting of 500 g/l glucose, 1% (w/v) $MgSO_4*7H2O$ and 2.2% (w/v) NH_4Cl) with 50 mg/l kanamycin.

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

For production of rubiwettin R1 with *E. coli* W3110 pACYC_rbwA_Srub as well as production of rubiwettin RG1 with *E. coli* W3110 pACYC_rbwAB_Srub and *E. coli* W3110 pACYC_rhlA_Pa rbwB_Srub, the three strains are cultured firstly from a cryoculture in LB medium (25 ml in a 100 ml baffled shake flask) with 50 mg/l kanamycin overnight at 37°C and 200 rpm for about 18 h. Then, 2 ml of this culture is transferred for a second preculture stage into 25 ml of high-cell-density medium consisting of $(NH_4)2SO_4$ 1.76 g/L, K_2HPO_4 19.08 g/l, KH_2PO_4 12.5 g/l, yeast extract 6.66 g/l, trisodium citrate dihydrate 11.2 g/l, 17 ml/l of a filter-sterilized 1% strength ammonium iron citrate solution, and 5 ml/l of a filter-sterilized trace element stock solution (consisting of HCl (37%) 36.50 g/l, $MnCl_2*4H_2O$ 1.91 g/l, $ZnSO_4*7H_2O$ 1.87 g/l, ethylenediaminetetraacetic acid dihydrate 0.84 g/l, H_3BO_3 0.30 g/l. $Na_2MoO_4*2H_2O$ 0.25 g/l, $CaCl_2*2H_2O$ 4.70 g/l, $FeSO_4*7H_2O$ 17.80 g/l, $CuCl_2*2H_2O$ 0.15 g/l) with 15 g/l glucose as carbon source (added by metered addition of 30 ml/l of a sterile

feed solution consisting of 500 g/l glucose, 1% (w/v) $MgSO_4*7H_2O$ and 2.2% (w/v) NH_4Cl) with the already described antibiotics in a 100 ml shake flask and incubated at 37°C/200 rpm for a further 6 h.

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

The standard program used is shown in Table 4:

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

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

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

Table 4. The standard program for production of rubiwettins with *E. coli* strains

The pH is regulated to pH 6.8 on one side with 12.5% strength ammonia solution. During cultivation and biotransformation, the dissolved oxygen (pO2 or DO) in the culture is regulated to at least 30% by means of stirrer feed and gassing rate. Following inoculation, the DO drops from 100% to this 30%, where it is kept stable for the remainder of the fermentation. The temperature is kept stable at 37°C.

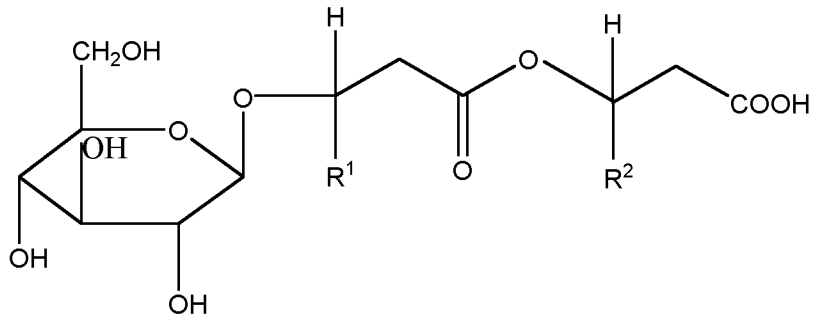
The fermentation is carried out as fed-batch, where the feed start is triggered as delivery to the feed phase with 5 g/l*h glucose feed, consisting of 500 g/l glucose, 1% (w/v) $MgSO_4*7H_2O$ and 2.2% (w/v) NH_4Cl, via the DO peak inducing the end of the batch phase. 3 h after the feed start,

rubiwettin production is induced with 0.2% (w/v) rhamnose. The inducer concentration refers to the volume at the beginning of fermentation. A rhamnose stock solution of 220 g/L is used. Quantification of formation of rubiwettins R1 and RG1 is performed as described in Example 7. It is shown that *E.coli* W3110 pACYC_rbwA_Srub produces rubiwettin R1.

It is also shown that *E. coli* W3110 pACYC_rbwAB_Srub and *E. coli* W3110 pACYC_rhlA_Pa rbwB_Srub both produce rubiwettins RG1.

CLAIMS

1. A microbial cell for producing at least one lipid with general formula II from at least one carbon substrate,



General Formula II

wherein R^1 and R^2 independently of one another comprises identical or different organic radicals each with 5 to 13 carbon atoms,
wherein the cell is a non-pathogenic cell that is genetically modified to increase the heterologous expression relative to the wild type cell of:
- an enzyme (E_2) capable of converting 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and/or 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) in combination with NDP-glucose into β-D-glucopyranosyl-3-hydroxyalkanoyl-3-hydroxyalkanoate.

2. The cell according to claim 1, wherein the enzyme E_2 is a glycosyltransferase (EC 2.4) comprising SEQ ID NO: 4 or variant thereof, wherein the variant comprises 60% sequence identity to SEQ ID NO:4.

3. The cell according to claim 1 or 2, wherein the R in the lipid with general formula II is a monounsaturated alkyl radical.

4. The cell according to claim 3, wherein the alkyl radical is selected from the group consisting of nonenyl, undecenyl and tridecenyl.

5. The cell according to any of the preceding claims, wherein the cell is further genetically modified to increase the heterologous expression relative to the wild type cell of:
   - an enzyme (E_1) capable of converting 3-hydroxyalkanoyl-CoA/ACP into 3-hydroxyalkanoyl-3-hydroxyalkanoyl-CoA/ACP and further to 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA).

6. The cell according to claim 5, wherein the enzyme E_1 is a 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) synthase.

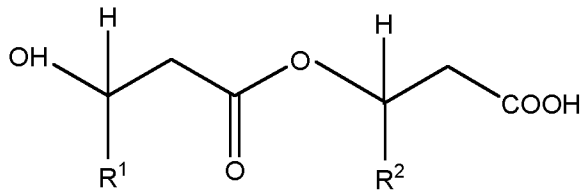
7. The cell according to either claim 5 or 6, wherein the enzyme E_1 comprises SEQ ID NO: 2 or variant thereof, wherein the variant comprises 60% sequence identity to SEQ ID NO:2.

8. The cell according to either claim 5 or 6, wherein the enzyme E_1 comprises a sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 and variants thereof, wherein the variant comprises 60% sequence identity to SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12 and SEQ ID NO: 14 respectively.

9. The cell according to any one of the preceding claims, wherein the cell is genetically modified to increase the expression of

- enzyme E_2 comprises SEQ ID NO: 4 or variant thereof, wherein the variant comprises 60% sequence identity to SEQ ID NO:4; and
- enzyme E_1 comprises SEQ ID NO: 2 or variant thereof, wherein the variant comprises 60% sequence identity to SEQ ID NO:2.

10. The cell according to any one of the preceding claims, wherein the cell produces a further lipid with general formula I from the carbon substrate,



General Formula I

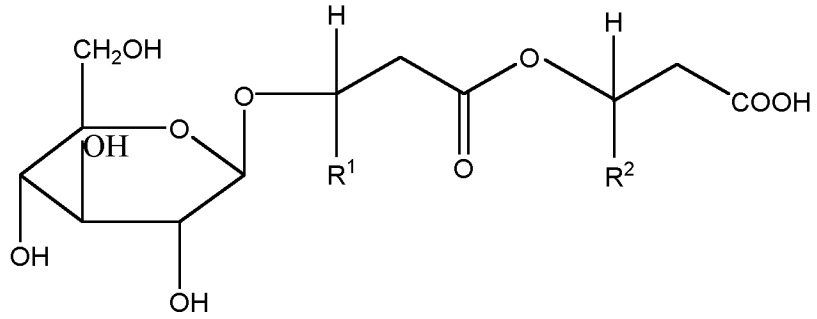
wherein R^1 and R^2 independently of one another comprises identical or different organic radicals each with 5 to 13 carbon atoms.

11. The cell according to claim 10, wherein the R in the lipid with general formula I is a monounsaturated alkyl radical.

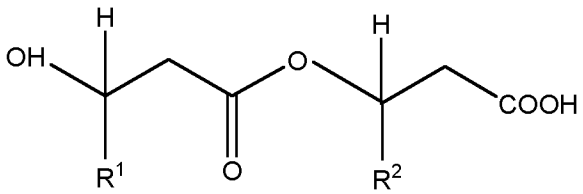
12. The cell according to any one of the preceding claims, wherein the carbon source is selected from the group consisting of glucose, dextrose, sucrose, polysaccharides, vegetal oils, animal fats, fatty acids, fatty acid esters, carbonaceous gases, alkanes, glycerol, acetate, ethanol and methanol.

13. The cell according to any one of the preceding claims, wherein the cell is selected from the group consisting of *Acinetobacter sp., Bacillus sp., Brevibacterium sp., Burkholderia sp., Chlorella sp., Clostridium sp., Corynebacterium sp., Cyanobakterien, Escherichia sp., Pseudomonas sp., Klebsiella sp., Salmonella sp., Rhizobium sp., Saccharomyces sp., Pichia sp.,* and *Nostoc sp.*.

14. A method of producing at least one lipid with general formula II and/ or general formula I:



General Formula II,

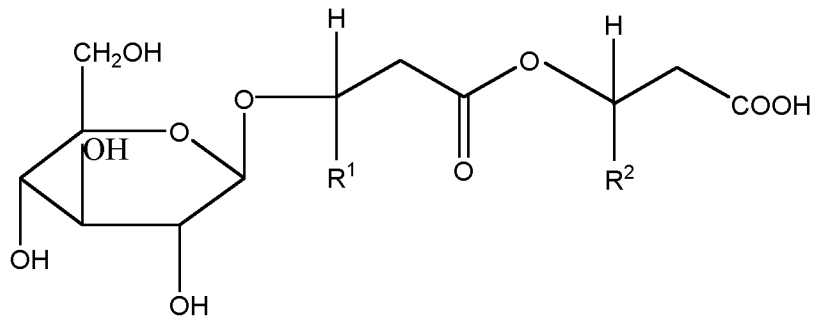


General Formula I

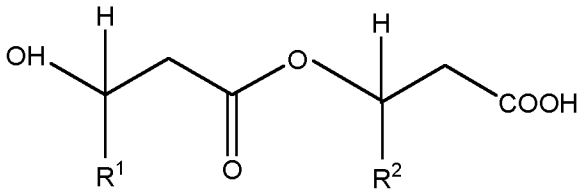
wherein R^1 and R^2 independently of one another comprises identical or different organic radicals each with 5 to 13 carbon atoms, and

wherein the method comprises a step of contacting at least one cell according to any one of the claims 1 to 13 with at least one carbon source.

15. Use of the cell according to any one of claims 1 to 13 for producing at least one lipid with general formula I and/or II:



General Formula II,



General Formula I

wherein R^1 and R^2 independently of one another comprises identical or different organic radicals each with 5 to 13 carbon atoms.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/053133

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/195 C12P19/18
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, Sequence Search, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	T MATSUYAMA ET AL: "Surface-active novel glycolipid and linked 3-hydroxy fatty acids produced by Serratia rubidaea.", JOURNAL OF BACTERIOLOGY, vol. 172, no. 6, 1 June 1990 (1990-06-01), pages 3015-3022, XP055469558, US ISSN: 0021-9193, DOI: 10.1128/jb.172.6.3015-3022.1990 page 3016 - page 3021; figure 12 -----	1-15
X	WO 2014/197457 A1 (US AGRICULTURE [US]) 11 December 2014 (2014-12-11) claims 1-66 ----- -/--	1-15

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 March 2019	15/03/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Seranski, Peter

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Uniprot: "AXX16_0219 -RhlB, TDP-rhamnosyltransferase 1 -Serratia rubidaea -AXX16_0219 gene & pr", From June, 8 June 2016 (2016-06-08), XP055470325, Retrieved from the Internet: URL:http://www.uniprot.org/uniprot/A0A126VCF31 of [retrieved on 2018-04-25] the whole document -----	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2019/053133

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014197457 A1	11-12-2014	US 2016102330 A1	14-04-2016
		WO 2014197457 A1	11-12-2014