

Claims

1. A yeast cell capable of producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol, said yeast cell expressing at least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA), wherein:
 - a) the at least one desaturase is Cpo_CPRQ (SEQ ID NO: 2), or a functional variant thereof having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity to SEQ ID NO: 2; or
 - b) the at least one desaturase is at least two desaturases, wherein at least one of said two desaturases is Cpo_CPRQ (SEQ ID NO: 2), or a functional variant thereof having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity to SEQ ID NO: 2, and the other desaturase is a desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 12, such as a Z9-12 desaturase.
2. The yeast cell according to claim 1, wherein the at least one desaturase is at least two desaturases, and wherein the other desaturase is selected from Cpo_NPVE (SEQ ID NO: 67), Cpo_SPTQ (SEQ ID NO: 69) and functional variants thereof having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity to SEQ ID NO: 67 or SEQ ID NO: 69.

3. The yeast cell according to any one of the preceding claims, wherein the yeast cell belongs to a genus selected from *Blakeslea*, *Candida*, *Cryptococcus*, *Cunninghamella*, *Lipomyces*, *Mortierella*, *Mucor*, *Phycomyces*, *Pythium*, *Rhodosporidium*, *Rhodotorula*, *Trichosporon*, *Saccharomyces* and *Yarrowia*, optionally wherein the yeast cell belongs to a species selected from *Blakeslea trispora*, *Candida pulcherrima*, *C. revkaufi*, *C. tropicalis*, *Cryptococcus curvatus*, *Cunninghamella echinulata*, *C. elegans*, *C. japonica*, *Lipomyces starkeyi*, *L. lipoferus*, *Mortierella alpina*, *M. isabellina*, *M. ramanniana*, *M. vinacea*, *Mucor circinelloides*, *Phycomyces blakesleanus*, *Pythium irregulare*, *Rhodosporidium toruloides*, *Rhodotorula glutinis*, *R. gracilis*, *R. graminis*, *R. mucilaginosa*, *R. pinicola*, *Trichosporon pullans*, *T. cutaneum*, *Saccharomyces cerevisiae* and *Yarrowia lipolytica*, preferably the yeast cell is a *Yarrowia lipolytica* cell or a *Saccharomyces cerevisiae* cell.
4. The yeast cell according to any one of the preceding claims, wherein the yeast cell is capable of producing *E8,E10*-dodecadien-1-ol, said yeast cell further expressing at least one heterologous fatty acyl-CoA reductase (EC 1.2.1.84) capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, wherein the fatty acyl-CoA reductase is capable of converting at least part of said *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA) to *E8,E10*-dodecadien-1-ol.
5. The yeast cell according to any one of the preceding claims, wherein the desaturase is a mutant of Cpo_CPRQ having a mutation at position 85, such as an S85A mutation, and/or wherein the at least one heterologous desaturase is at least two different heterologous desaturases, such as Cpo_CPRQ as set forth in SEQ ID NO: 2 and a mutant of Cpo_CPRQ having a mutation at position 85 such as an S85A mutation.
6. The yeast cell according to any one of claims 4 to 5, wherein the fatty acyl-CoA reductase is selected from the group consisting of Ase_FAR (SEQ ID NO: 10), Aip_FAR (SEQ ID NO: 61), Hs_FAR (SEQ ID NO: 71), Has_FAR (SEQ ID NO: 73), Hv_FAR (SEQ ID NO: 75), Har_FAR (SEQ ID NO: 12), Cpo_FAR (SEQ ID NO: 76) and functional variants thereof having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% r identity thereto, optionally wherein the fatty acyl-CoA

reductase is a mutant of Ase_FAR, such as having a mutation at position 198 or 413, preferably a T198A mutation or an S413A mutation.

7. The yeast cell according to any one of the preceding claims, further having one or more of the following:

- expressing a heterologous cytochrome b5, such as a cytochrome b5 from a *Lepidoptera* species, such as a cytochrome b5 from *Helicoverpa armigera*, preferably the cytochrome b5 HarCyb5 as set forth in SEQ ID NO: 4 or a functional variant thereof having at least 80% identity thereto, such as at least 85%, such as at least 90%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity thereto;
- expressing a heterologous cytochrome b5 reductase (EC 1.6.2.2), such as a cytochrome b5 reductase from a *Lepidoptera* species, such as *Helicoverpa armigera*, preferably the cytochrome b5 reductase is the cytochrome b5 reductase from *Helicoverpa armigera* as set forth in SEQ ID NO: 24 or a functional variant thereof having at least 80% identity thereto, such as at least 85%, such as at least 90%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity thereto,
- expressing a hemoglobin, such as a hemoglobin from *Vitreoscilla stercoraria*, preferably the hemoglobin from *Vitreoscilla stercoraria* as set forth in SEQ ID NO: 6 or a functional variant thereof having at least 80% identity thereto, such as at least 85%, such as at least 90%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity thereto,
- comprising a mutation of one or more genes encoding an elongase and resulting in a partial or total loss of elongase activity, such as a mutation of the *ELO1* gene (SEQ ID NO: 13) resulting in a partial or total loss of Elo1 activity, preferably wherein said mutation is a deletion,
- comprising a mutation of one or more genes encoding a thioesterase and resulting in a partial or total loss of thioesterase activity, such as a mutation of the *YAL10_F14729g* gene (SEQ ID NO: 19), a mutation of the *YAL10_E18876g* gene (SEQ ID NO: 54) or a mutation of *YAL10_D03597g* (SEQ ID NO: 55), preferably wherein said mutation is a deletion,
- comprising at least one mutation resulting in reduced activity of at least one of Hfd1, Hfd2, Hfd3, Hfd4, Fao1 and Pex10, or having a mutation resulting in reduced activity of at least one protein having at least 80% identity thereto, such as at least 81%, such as

- at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity thereto,
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- expressing a fatty acyl synthase variant having a modified ketone synthase domain, wherein said fatty acyl synthase variant is a variant of Fas1 (SEQ ID NO: 16) or Fas2 (SEQ ID NO: 18) such as a mutant Fas1 having a mutation at position 123, preferably an L123V mutation, or a mutant Fas2 having a mutation at position 1220, preferably an
 - 10 I1220F or an I1220W mutation,
 - expressing a thioesterase such as a heterologous thioesterase, optionally wherein the thioesterase is expressed at high level, such as a thioesterase having at least 80% identity to the thioesterase from *Cuphea palustris* as set forth in SEQ ID NO: 33, to the thioesterase from *Cuphea hookeriana* as set forth in SEQ ID NO: 57, to the thioesterase
 - 15 from *Cinnamomum camphora* as set forth in SEQ ID NO: 35, or to the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 26, preferably the thioesterase has at least 80% identity to the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 35, or to the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 26,
 - expressing a fusion protein of a truncated fatty acyl synthase and of a truncated
 - 20 thioesterase, such as the fusion protein as set forth in SEQ ID NO: 59 or a homologue thereof having at least 80% identity thereto.
8. The yeast cell according to any one of the preceding claims, further comprising at least one mutation resulting in reduced activity of at least one of Hfd1, Hfd2, Hfd3, Hfd4,
- 25 Fao1, GPAT and Pex10, or having at least one mutation resulting in reduced activity of at least one protein having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as
- 30 at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity thereto.
9. The yeast cell according to any one of the preceding claims, wherein the yeast cell is capable of producing *E8,E10*-dodecadien-1-ol with a titer of at least 0.5 mg/L, such as at
- 35 least 0.6 mg/L, such as at least 0.7 mg/L, such as at least 0.8 mg/L, such as at least 0.9 mg/L, such as at least 1 mg/L, such as at least 1.5 mg/L, such as at least 2.5 mg/L, such

as at least 5.0 mg/L, such as at least 10 mg/L, such as at least 15 mg/L, such as at least 20 mg/L, such as 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 750 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, such as at least 6 g/L, such as at least 7 g/L, such as at least 8 g/L, such as at least 9 g/L, such as at least 10 g/L or more.

10. The yeast cell according to any one of the preceding claims, wherein the yeast cell further expresses an acetyltransferase (EC 2.3.1.84) capable of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienyl acetate, whereby the yeast cell is capable of producing *E8,E10*-dodecadienyl acetate, preferably wherein the acetyltransferase is a heterologous acetyltransferase (AcT) expressed from said yeast cell or a native acetyltransferase overexpressed from said yeast cell, preferably wherein the acetyltransferase is Sc_At1f1 (SEQ ID NO: 37) or a functional variant thereof having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity to Sc_At1f1 (SEQ ID NO: 37).

11. The yeast cell according to any one of the preceding claims, wherein the yeast cell further expresses an aldehyde-forming fatty acyl-CoA reductase (EC 1.2.1.50), an alcohol dehydrogenase (EC 1.1.1.2) and/or a fatty alcohol oxidase (EC 1.1.3.20) capable of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienal.

12. The yeast cell according to any one of the preceding claims, wherein the yeast cell further:

- i) has one or more mutations resulting in reduced activity of one or more native acyl-CoA oxidases; and
- ii) expresses at least one group of enzymes comprising at least one acyl-CoA oxidase capable of oxidising a fatty acyl-CoA, wherein the group of enzymes is capable of shortening a fatty acyl-CoA of a first carbon chain length X to a shortened fatty acyl-CoA having a second carbon chain length X' , wherein $X' \leq X-2$, preferably wherein $X'=12$.

13. The yeast cell according to any one of the preceding claims, further expressing a desaturase capable of introducing at least one double bond in the fatty acyl-CoA of carbon chain length X, such as CroZ11 desaturase (SEQ ID NO: 63) or CpaE11 desaturase (SEQ ID NO: 65), or a functional variant thereof having at least 80% identity, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity to SEQ ID NO: 63 or SEQ ID NO: 65.
14. A method for producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol in a yeast cell, said method comprising the steps of providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell expresses:
- At least one heterologous desaturase capable of introducing one or more double bonds in a fatty acyl-CoA having a carbon chain length of 12, thereby converting said fatty acyl-CoA to a desaturated fatty acyl-CoA, wherein at least part of said desaturated fatty acyl-CoA is *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA); wherein:
 - the at least one desaturase is Cpo_CPRQ (SEQ ID NO: 2), or a functional variant thereof having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% identity to SEQ ID NO: 2; or
 - the at least one desaturase is at least two desaturases, wherein at least one of said two desaturases is Cpo_CPRQ (SEQ ID NO: 2), or a functional variant thereof having at least 80% identity thereto, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at

least 99% identity to SEQ ID NO: 2, and the other desaturase is a desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 12, such as a Z9-12 desaturase;

and

- 5 ii) Optionally at least one heterologous fatty acyl-CoA reductase (EC 1.2.1.84) capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, wherein the fatty acyl-CoA reductase is capable of converting at least part of said *E8,E10*-dodecadienyl coenzyme A (*E8,E10*-C12:CoA) to *E8,E10*-dodecadien-1-ol,

10 thereby producing *E8,E10*-dodecadienyl coenzyme A and optionally *E8,E10*-dodecadien-1-ol.

15 15. The method according to claim 14, wherein the method further comprises the steps of converting the *E8,E10*-dodecadienyl coenzyme A into a lipid such as a triacylglyceride or into a free fatty acid, recovering said lipid or free fatty acid and converting said lipid or free fatty acid to *E8,E10*-dodecadien-1-ol.

20 16. The method according to any one of claims 14 to 15, wherein the method further comprises the step of recovering said *E8,E10*-dodecadien-1-ol, preferably wherein the yeast cell is as defined in any one of claims 1 to 13.

25 17. The method according to any one of claims 14 to 16, further comprising the step of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienyl acetate by expression of an acetyltransferase or by chemical conversion, thereby further producing *E8,E10*-dodecadienyl acetate, and optionally further comprising the step of recovering said *E8,E10*-dodecadienyl acetate.

30 18. The method according to any one of claims 14 to 17, further comprising the step of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienal by expression of an aldehyde-forming fatty acyl-CoA reductase (EC 1.2.1.50), an alcohol dehydrogenase (EC 1.1.1.2) and/or a fatty alcohol oxidase (EC 1.1.3.20) capable of converting at least part of the *E8,E10*-dodecadien-1-ol into *E8,E10*-dodecadienal, or by chemical conversion, thereby further producing *E8,E10*-dodecadienal, and optionally further comprising the step of recovering said *E8,E10*-dodecadienal.

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19. The method according to any one of claims 14 to 18, wherein the medium comprises an extractant in an amount equal to or greater than its cloud concentration in an aqueous solution such as in the culture medium at the cultivation temperature, wherein the extractant a non-ionic ethoxylated surfactant such as an antifoaming agent, preferably a polyethoxylated surfactant selected from: a polyethylene polypropylene glycol, a mixture of polyether dispersions, an antifoaming agent comprising polyethylene glycol monostearate such as simethicone, fatty alcohol alkoxylates, polyethoxylated surfactants and ethoxylated and propoxylated C₁₆-C₁₈ alcohol-based antifoaming agents and combinations thereof.
20. The method according to claim 19, wherein the medium comprises the extractant in an amount greater than its cloud concentration by at least 50%, such as at least 100%, such as at least 150%, such as at least 200%, such as at least 250%, such as at least 300%, such as at least 350%, such as at least 400%, such as at least 500%, such as at least 750%, such as at least 1000%, or more, and/or wherein the culture medium comprises the extractant in an amount at least 2-fold its cloud concentration, such as at least 3-fold its cloud concentration, such as at least 4-fold its cloud concentration, such as at least 5-fold its cloud concentration, such as at least 6-fold its cloud concentration, such as at least 7-fold its cloud concentration, such as at least 8-fold its cloud concentration, such as at least 9-fold its cloud concentration, such as at least 10-fold its cloud concentration, such as at least 12.5-fold its cloud concentration, such as at least 15-fold its cloud concentration, such as at least 17.5-fold its cloud concentration, such as at least 20-fold its cloud concentration, such as at least 25-fold its cloud concentration, such as at least 30-fold its cloud concentration, wherein the cloud concentration is measured in the medium, preferably at the cultivation temperature.
21. The method according to any one of claims 19 to 20, further comprising the step of converting the *E8,E10*-dodecadienyl coenzyme A into a lipid or a free fatty acid, and wherein said lipid or free fatty acid, said *E8,E10*-dodecadien-1-ol, and optionally said *E8,E10*-dodecadienyl acetate and/or said *E8,E10*-dodecadienal produced by the yeast cell is present in an emulsion in the fermentation broth, the method further comprising a step of breaking said emulsion, thereby obtaining a composition comprising a product phase comprising the extractant and the lipid or free fatty acid, the *E8,E10*-dodecadien-1-ol, and optionally the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal.

22. The method according to claim 21, wherein the step of breaking the emulsion comprises or consists of a step of phase separation, such as a step of centrifugation, of the fermentation broth, thereby obtaining a composition consisting of three phases: a water phase, a phase comprising cells and cellular debris, and the product phase comprising the extractant and the lipid or free fatty acid, *E8,E10*-dodecadien-1-ol, and optionally the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal.
23. The method according to any one of claims 21 to 22, wherein the product phase comprises at least 50% of the lipid or free fatty acid, *E8,E10*-dodecadien-1-ol, and optionally of the *E8,E10*-dodecadienyl acetate and/or of the *E8,E10*-dodecadienal initially present in the fermentation broth, such as at least 55%, such as at least 60%, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 95% or more.
24. The method according to any one of claims 21 to 23, further comprising the steps of:
- recovering the lipid or free fatty acid, the *E8,E10*-dodecadien-1-ol, and optionally the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal preferably by a distillation step such as a distillation under reduced pressure, or by a column purification,
 - chemically converting at least part of the *E8,E10*-dodecadien-1-ol to *E8,E10*-dodecadienal and/or to *E8,E10*-dodecadienyl acetate,
 - optionally, recovering said *E8,E10*-dodecadienal and/or to *E8,E10*-dodecadienyl acetate.
25. The method according to any one of claims 14 to 24, further comprising the step of formulating the recovered *E8,E10*-dodecadien-1-ol, the *E8,E10*-dodecadienyl acetate and/or the *E8,E10*-dodecadienal into a pheromone composition.