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CANADIAN PATENT APPLICATION**

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(71) Demandeur/Applicant:  
CORBION BIOTECH, INC., US

(72) Inventeurs/Inventors:  
FRANKLIN, SCOTT, US;  
MOSELEY, JEFFREY L., US;  
RAKITSKY, WALT, US;  
RUDENKO, GEORGE, US;  
SOMANCHI, ARAVIND, US;

(54) Titre : HUILES SPECIFIQUES PRODUITES A PARTIR DE MICROORGANISMES HETEROTROPHES  
RECOMBINANTS

(54) Title: TAILORED OILS PRODUCED FROM RECOMBINANT HETEROTROPHIC MICROORGANISMS

(57) **Abrégé/Abstract:**

Methods and compositions for the production of oil, fuels, oleochemicals, and other compounds in recombinant microorganisms are provided, including oil-bearing microorganisms and methods of low cost cultivation of such microorganisms. Microalgal cells containing exogenous genes encoding, for example, a lipase, a sucrose transporter, a sucrose invertase, a fructokinase, a polysaccharide-degrading enzyme, a keto acyl-ACP synthase enzyme, a fatty acyl- ACP thioesterase, a fatty acyl-CoA/aldehyde reductase, a fatty acyl-CoA reductase, a fatty aldehyde reductase, a fatty aldehyde decarbonylase, and/or an acyl carrier protein are useful in manufacturing transportation fuels such as renewable diesel, biodiesel, and renewable jet fuel, as well as oleochemicals such as functional fluids, surfactants, soaps and lubricants.

(72) Inventeurs(suite)/Inventors(continued): WEE, JANICE, US

(74) Agent: SMART & BIGGAR

**ABSTRACT**

Methods and compositions for the production of oil, fuels, oleochemicals, and other compounds in recombinant microorganisms are provided, including oil-bearing microorganisms and methods of low cost cultivation of such microorganisms. Microalgal cells containing exogenous genes encoding, for example, a lipase, a sucrose transporter, a sucrose invertase, a fructokinase, a polysaccharide-degrading enzyme, a keto acyl-ACP synthase enzyme, a fatty acyl-ACP thioesterase, a fatty acyl-CoA/aldehyde reductase, a fatty acyl-CoA reductase, a fatty aldehyde reductase, a fatty aldehyde decarbonylase, and/or an acyl carrier protein are useful in manufacturing transportation fuels such as renewable diesel, biodiesel, and renewable jet fuel, as well as oleochemicals such as functional fluids, surfactants, soaps and lubricants.

## CLAIMS:

1. A microalgal triglyceride oil derived from microalgae and having a fatty acid profile comprising at least 60% C18:1 and less than 7% C18:2, or at least 35% saturated fatty acids, wherein the microalgae comprise an inactivated or mutated endogenous desaturase with less enzymatic activity or an exogenous gene encoding a fatty acyl-ACP thioesterase.
2. The oil of claim 1 having a fatty acid profile comprising at least 60% C18:1 and less than 7% C18:2, wherein the microalgae comprise an inactivated or mutated endogenous desaturase with less enzymatic activity.
3. The oil of claim 2, wherein the endogenous desaturase is inactivated by an exogenous gene via homologous recombination.
4. The oil of any of claims 1 to 3, wherein the microalgae further comprises an exogenous gene that encodes a sucrose invertase.
5. The oil of any one of claims 1 to 4 that comprises at least 70%, at least 75%, or at least 80% C18:1.
6. The oil of any one of claims 1 to 5 that comprises less than 5%, less than 3%, or less than 1% C18:2.
7. The oil of claim 1 having a fatty acid profile comprising at least 35% saturated fatty acids.
8. The oil of claim 1 or 7 that comprises at least 65% saturated fatty acids.
9. The oil of claim 8 that comprises at least 80%, at least 85%, or at least 90% saturated fatty acids.
10. The oil of any one of claims 1 to 9, wherein the fatty acid profile further comprises one or more of at least 1% C8:0, at least 1% C10:0, at least 1% C12:0, at least 2% C14:0, at least 30% C16:0, and at least 5% C18:0.



11. The oil of any one of claims 1 to 10, wherein the oil comprises a plurality of sterols derived from the microalgae.

12. The oil of any one of claims 1 to 11, wherein the oil comprises less than 1% betasitosterol.

13. The oil of any one of claims 1 to 12, wherein the microalgae are of the genus *Prototheca*.

14. The oil of claim 13, wherein the microalgae are *Prototheca moriformis*.

15. A product prepared from the oil of any of claim 1 to 14, wherein the oil is subjected to at least one chemical reaction selected from the group consisting of: saponification; metathesis; acid hydrolysis; alkaline hydrolysis; enzymatic hydrolysis; catalytic hydrolysis; hot-compressed water hydrolysis; a catalytic hydrolysis reaction wherein the lipid is split into glycerol and fatty acids; an amination reaction to produce fatty nitrogen compounds; an ozonolysis reaction to produce mono- and dibasic-acids; a triglyceride splitting reaction selected from the group consisting of enzymatic splitting and pressure splitting; a condensation reaction that follows a hydrolysis reaction; a hydroprocessing reaction; a hydroprocessing reaction and a deoxygenation reaction or a condensation reaction prior to or simultaneous with the hydroprocessing reaction; a gas removal reaction; a deoxygenation reaction selected from the group consisting of a hydrogenolysis reaction, hydrogenation, a consecutive hydrogenation-hydrogenolysis reaction, a consecutive hydrogenolysis-hydrogenation reaction, and a combined hydrogenation-hydrogenolysis reaction; a condensation reaction following a deoxygenation reaction; an esterification reaction; an interestification reaction; a transesterification reaction; a hydroxylation reaction; and a condensation reaction following a hydroxylation reaction.

16. A food composition comprising the oil of any one of claims 1 to 14 together with one or more other edible ingredients.

17. The food composition of claim 16, that is a salad dressing, egg product, baked good, bread, bar, snack chip, pasta, sauce, soup, beverage, frozen dessert, butter or spread.

18. A method of making a food composition comprising combining the oil of any one of claims 1 to 14 with at least one other edible ingredient.
19. The method of claim 18, further comprising:
- a. determining the amount of non-algal oil, non-algal fat or egg in a conventional food product; and
  - b. replacing a portion of the non-algal oil, non-algal fat or egg or supplementing the non-algal oil, non-algal fat or egg with a specified amount of the microalgal triglyceride oil.
20. The method of claim 18 or claim 19, wherein non-algal oil, non-algal fat or egg is not added to the food composition.
21. The method of claim 19 or claim 20, wherein the amount of microalgal triglyceride oil is from 0.25 times to 4 times the mass or volume of the non-algal oil, non-algal fat or egg in the conventional food product.
22. A method of producing a microalgal triglyceride oil according to any one of claims 1 to 14, the method comprising:
- cultivating a population of microalgal cells in a culture medium; and
  - isolating the microalgal triglyceride oil from the microalgal cells.
23. A recombinant microalgal cell that produced triglyceride oil, said triglyceride oil having a fatty acid profile comprising at least 60% C18:1 and less than 7% C18:2, or at least 35% saturated fatty acids, wherein the microalgae comprise an inactivated or mutated endogenous desaturase with less enzymatic activity or an exogenous gene encoding a fatty acyl-ACP thioesterase.
24. The cell of claim 23, wherein the microalgae comprise an inactivated or mutated endogenous desaturase with less enzymatic activity.
25. The cell of claim 24, wherein the endogenous desaturase is inactivated by an exogenous gene via homologous recombination.



26. The cell of any of claims 23 to 25, wherein the microalgae further comprises an exogenous gene that encodes a sucrose invertase.

27. The cell of any one of claims 23 to 26, wherein the triglyceride oil comprises at least 70%, at least 75%, or at least 80% C18:1.

28. The cell of any one of claims 23 to 27, wherein the triglyceride oil comprises less than 5%, less than 3%, or less than 1% C18:2.

29. The cell of any of claims 23 to 28, wherein the triglyceride oil has a fatty acid profile comprising at least 35% saturated fatty acids.

30. The cell of claim 23 or 29, wherein the triglyceride oil comprises at least 65% saturated fatty acids.

31. The cell of claim 30, where in the triglyceride oil comprises at least 80%, at least 85%, or at least 90% saturated fatty acids.

32. The cell of any one of claims 23 to 31, wherein the fatty acid profile of the triglyceride oil further comprises one or more of at least 1% C8:0, at least 1% C10:0, at least 1% C12:0, at least 2% C14:0, at least 30% C16:0, and at least 5% C18:0.

33. The cell of any one of claims 23 to 32, wherein the oil comprises a plurality of sterols derived from the microalgae.

34. The cell of any one of claims 23 to 33, wherein the oil comprises less than 1% betasitosterol.

35. The cell of any one of claims 23 to 34, wherein the microalgae are of the genus *Prototheca*.

36. The cell of claim 35, wherein the microalgae are *Prototheca moriformis*.

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Figure 1

