

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PCT25752FZRPpau	FOR FURTHER ACTION		See Form PCT/IPEA/416
International application No. PCTUS2015/036078	International filing date (day/month/year) 16.06.2015	Priority date (day/month/year) 16.06.2014	
International Patent Classification (IPC) or national classification and IPC INV. C12N9/02			
Applicant REG LIFE SCIENCES, LLC.			
1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36. 2. This REPORT consists of a total of <u>6</u> sheets, including this cover sheet. 3. This report is also accompanied by ANNEXES, comprising: a. ☒ (sent to the applicant and to the International Bureau) a total of <u>9</u> sheets, as follows: ☒ sheets of the description, claims and/or drawings which have been amended and/or sheets containing rectifications authorized by this Authority, unless those sheets were superseded or cancelled, and any accompanying letters (see Rules 46.5, 66.8, 70.16, 91.2, and Section 607 of the Administrative Instructions). ☐ sheets containing rectifications, where the decision was made by this Authority not to take them into account because they were not authorized by or notified to this Authority at the time when this Authority began to draw up this report, and any accompanying letters (Rules 66.4bis, 70.2(e), 70.16 and 91.2). ☐ superseded sheets and any accompanying letters, where this Authority either considers that the superseding sheets contain an amendment that goes beyond the disclosure in the international application as filed, or the superseding sheets were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in item 4 of Box No. I and the Supplemental Box (see Rule 70.16(b)). b. ☐ (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing, in the form of an Annex C/ST.25 text file, as indicated in the Supplemental Box Relating to Sequence Listing (see paragraph 3ter of Annex C of the Administrative Instructions).			
4. This report contains indications relating to the following items: ☒ Box No. I Basis of the report ☐ Box No. II Priority ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability ☐ Box No. IV Lack of unity of invention ☒ Box No. V Reasoned conclusion under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement ☒ Box No. VI Certain documents cited ☐ Box No. VII Certain defects in the international application ☐ Box No. VIII Certain observations on the international application			
Date of submission of the demand 18.04.2016		Date of completion of this report 01.06.2016	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465		Authorized officer Chavanne, Franz Telephone No. +49 89 2399-8399	



INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US2015/036078

Box No. I Basis of the report

1. With regard to the **language**, this report is based on
- ☒ the international application in the language in which it was filed
 - ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
 - ☐ international search (under Rules 12.3(a) and 23.1(b))
 - ☐ publication of the international application (under Rule 12.4(a))
 - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a) and (b))
2. With regard to the **elements*** of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

Description, Pages

1-94 as originally filed

Claims, Numbers

1-44 filed in electronic form on

18-04-2016

Drawings, Sheets

1/2, 2/2 as originally filed

- ☒ a sequence listing - see Supplemental Box Relating to Sequence Listing.
3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and (c-bis)):
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
5. ☐ This report has been established:
- ☐ taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rules 66.1(d-bis) and 70.2(e)).
 - ☐ without taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91(Rules 66.4bis and 70.2(e)).

**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
PCT/US2015/036078

6. ☒ With regard to top-up searches (Rules 66.1 *ter* and 70.2(f)):
- ☒ A top-up search was carried out by this Authority on 13.05.2016 (all discovered documents are listed in the Supplemental Box Relating to Top-up Search).
 - ☐ Additional relevant documents have been discovered during the top-up search.
 - ☐ No top-up search was carried out by this Authority because it would serve no useful purpose.
7. ☐ Supplementary international search report(s) from Authority(ies) has/have been received and taken into account in establishing this report (Rule 45bis.8(b) and (c)).

* If item 4 applies, some or all of those sheets may be marked "superseded".

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	<u>1-44</u>
	No: Claims	
Inventive step (IS)	Yes: Claims	<u>1-44</u>
	No: Claims	
Industrial applicability (IA)	Yes: Claims	<u>1-44</u>
	No: Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of a sequence listing:
 - a. ☐ forming part of the international application as filed:
 - ☐ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☒ furnished subsequent to the international filing date for the purposes of international search and/or examination:
 - ☒ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
 - d. ☐ furnished to this Authority as an amendment* under PCT Article 34 on :
 - ☐ in the form of an Annex C/ST.25 text file, and preferably identified as "Amended" at the first line of text.
 - ☐ on paper or in the form of an image file.
2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the sequence listing, which forms part of the basis of the report, may be marked "superseded."*

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following documents:

- D1 DANIEL SCHEPS ET AL: "Synthesis of [omega]-hydroxy dodecanoic acid based on an engineered CYP153A fusion construct", MICROBIAL BIOTECHNOLOGY, 1 August 2013 (2013-08-01), pages n/a-n/a, XP55115867, ISSN: 1751-7915, DOI: 10.1111/1751-7915.12073
- D2 WO 2014/201474 A1 (LS9 INC [US]; SCHIRMER ANDREAS W [US]; WANG HAIBO [US]; DEL CARDAYRE S) 18 December 2014 (2014-12-18)
- D3 SUMIRE HONDA MALCA ET AL: "Bacterial CYP153A monooxygenases for the synthesis of omega-hydroxylated fatty acids", CHEMICAL COMMUNICATIONS - CHEMCOM; [6015D], ROYAL SOCIETY OF CHEMISTRY, GB, [Online] vol. 48, 1 March 2012 (2012-03-01), pages 5115-5117, XP002695533, ISSN: 1359-7345, DOI: 10.1039/C2CC18103G
Retrieved from the Internet:
URL:<http://pubs.rsc.org/en/Content/ArticleLanding/2012/CC/c2cc18103g>
[retrieved on 2012-03-26]
cited in the application
- D4 EP 2 639 308 A1 (EVONIK INDUSTRIES AG [DE]) 18 September 2013 (2013-09-18)

2 The subject-matter of claims 1-44 is not explicitly describes in the prior art. The claimed subject-matter is, thus, novel (Article 33(2) PCT).

3 The closest prior art to evaluate the inventiveness of the present application is D1.

D1 describes CYP153A-reductase fusion proteins, including one comprising the CYP153A of SEQ ID No.6 of the present application. The fusion proteins of D1 yield omega-hydroxy dodecanoic acid from dodecanoic acid or the corresponding methyl ester (abstract; pages 695-704).

The subject-matter of claims 1-44 differs from the teaching of D1 in that D1 does not mention that the CYP153A monooxygenase of SEQ ID No.6 comprised in the hybrid-fusion protein further comprises at least one mutation.

In view of D1, the problem to be solved by the present application was to provide a further CYP153A-reductase fusion protein with improved catalytic activity conversion of a fatty acid to an omega-hydroxylated fatty acid.

The solution provided by the present application is the introduction of at least one mutation at position 796, 141, 27, 178, 309, 407 or 415.

The G307A mutated form of the CYP153A-reductase is known in the art (see e.g. D1, D3). The mutation G307A is present in the catalytically active site close to the heme group of the globular protein CYP153A. The catalytic activity of this mutant is improved as compared to the non-mutated CYP153A. The present application refers, however, to further substitutions of residues located outside the catalytic site of the protein. It could not be expected that mutations outside the catalytic site of the protein would result in protein having further improved catalytic activity.

The subject-matter of claims 1-44 is, thus, inventive (Article 33(3)PCT).

Re Item VI

Certain documents cited

- 1 WO 2014/201474

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Ihr Zeichen / Your Ref.

Unser Zeichen / Our Ref.

Datum / Date

PCT25752FZRPpau

April 18, 2016

International Patent Application PCT/US2015/036078
Applicant: REG Life Sciences, LLC

This letter is accompanying the Chapter II Demand for International Preliminary Examination:

Submitted herewith are amended claims under Article 34 PCT, which should form the basis for International Preliminary Examination.

1. AMENDMENTS TO THE CLAIMS

New claims 1-44 correspond to original PCT claims 1-12 and 16-47, except for omitting reference to amino acid positions 82, 231, 516, and 666 from new claim 1. Original claims 13-15 have been omitted from the set of claims accordingly, and the dependent claims have been adapted in accordance with the amendment made to claim 1.

The amendments made to the claims do not introduce subject matter, which extends beyond the disclosure content of the application as originally filed.

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2. NOVELTY (ARTICLE 33(2) PCT)

As correctly stated in section 2 of the written opinion of the International Searching Authority, the subject matter of claims 1-47 is novel in accordance with Article 33(3) PCT.

3. INVENTIVE STEP (ARTICLE 33(3) PCT)

In section 3 of the written opinion, it is held that the claimed subject matter lacks an inventive step in view of cited reference D1 (Scheps *et al.* 2013).

In particular, it is held that the claimed mutations were obvious for the skilled person when aiming at providing further CYP153A-reductase fusion proteins.

We respectfully disagree, as explained in the following:

D1 describes CYP153A-reductase fusion proteins (see Table 1 of D1) that are based on native CYP153A and a mutated form thereof, namely CYP153A carrying a mutation at position 307 (G307A).

According to the written opinion, the difference between the subject matter of present claims 1-47 and D1 is that the hybrid fusion protein of the present invention "*further comprises at least one mutation*".

However, and this is important to note, claim 1 does not merely refer in broad terms to "at least one further mutation", but describes that the at least one further mutation is at a selected position comprising positions 27, 141, 178, 309, 407, 415, and 796.

The claimed subject matter was not obvious in view of D1 when taking into account the fundamental difference between the claimed positions and position 307 of the hybrid fusion protein of D1.

The identification and characterization of the CYP153A_(G307A) mutant is described in cited reference D3 (Honda-Malca *et al.* 2012¹).

¹ The research group of D1 and D3 is the same. Note that the path forward taken by the group was not to generate further mutants, but to make fusion constructs of CYP153A and the CYP153A_(G307A) mutant containing different types of C-terminally linked reductase domains (see Fig. 1 and Table 1 of D1). D1 basically characterizes the CYP153A with different reductase fusion partners while modifying the length of the linker domain.

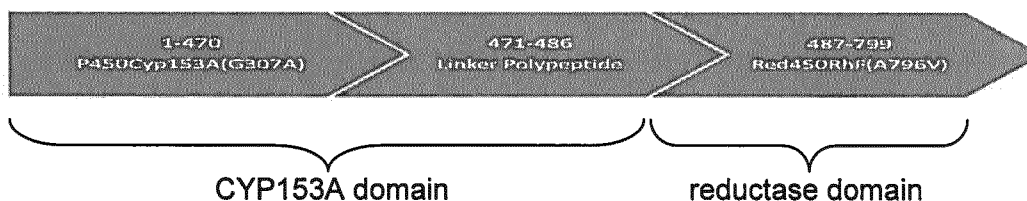
As explained in D3 at page 5116, left column, 1st sentence of 2nd paragraph, the authors were looking for "*substrate-interacting residues in CYP153A*". To this end, they performed a structure-based analysis focusing on the identification of key residues in structural elements that

"contain amino acids pointing with their side chains towards the heme center and are hence expected to be in contact with every substrate molecule during the attack of the activated oxygen."

(D1, p. 5116, left col., 2nd para., emphasis added)

As further described in this text passage, position G307 is one of the two "*hotspot positions, which can be easily identified from the protein sequence*", and which is "*also part of the active site of a previously published homology model built for CYP153A6*" (emphasis added).

Contrast this approach with the one taken by the inventors of the present application, which is not a structure-based approach focusing on the active site of the CYP153A domain. Rather, the inventors generated entirely random mutations in an effort to improve product output (see page 85, paragraph [0164], and the saturation libraries in Tables 5-11 of the application as originally filed). None of positions 27, 141, 178, 309, 407, 415, and 796 described in new claim 1 is located in the active site of the CYP153A domain. Furthermore, position 796 is not even located in the CYP153A catalytic domain, but is located in the reductase domain, as depicted in the following figure:



As a matter of fact, CYP153A is a globular protein and its catalytically active site involves the amino acid residues that are adjacent to the heme group (where actually the chemistry of substrate hydroxylation occurs). Based on the structural information of CYP153A, the research group of D1/D3 was investigating position G307 as one of the positions that are in close proximity to the heme in the active site.

The inventors of the present application have surprisingly found that the catalytic properties of CYP153A can be improved when mutating specific positions that are not necessarily in the active site of the protein. For example, the specific mutations in the CYP153A domain which provide for an improved catalytic activity, positions 27, 141, 178, 309, 407, and 415 as described in claim 1, are not located in the active site of the CYP153A domain based 3-D structural modeling.

The enclosed 3D-structure model of CYP153A shows the heme group (as stick model) with the iron atom in orange in the middle, and the proposed ligand binding site (active site) is depicted by the light grey surface. The mutated residues are depicted as sphere models (light blue, dark blue and red describe carbon atom, nitrogen atom, and oxygen atom, respectively, on the mutated residues). The structure model is portrayed in four different views showing a rotation in space in order to visualize that the claimed mutations are not in the active site.

The application as filed contains data showing the improved properties of the claimed mutants. In particular, Tables 6 and 7 on page 86-88 of the specification show an improvement in ω -hydroxylase activity (increased ω -OH fatty acid titer) for mutations 27, 141, 178, 309, 407, and 415, while Table 8 on page 89 shows an improvement in ω -hydroxylase activity for mutation 796.

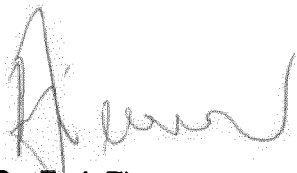
It is respectfully submitted that neither D1 nor D3 teach or suggest that a mutation outside the active center of CYP153A, let alone a mutation at one of the specific positions 27, 141, 178, 309, 407, and 415, would (not just **could**, but **would**) provide for an improvement in biological activity of the protein. The same holds for position 796 in the reductase domain of the claimed hybrid fusion protein.

Therefore, the claimed subject matter was not obvious for the person skilled in the art in view of cited references D1 and D3.

New claims 1-44 submitted herewith involve an inventive step in accordance with Article 33(3) PCT.

4. REQUESTS

The European Patent Office acting as International Searching Authority is kindly requested to carry out the International Preliminary Examination based on the new set of claims submitted herewith and taking into account the above explanations in support of inventive step.



Dr. F.-J. Zimmer
- Patent Attorney –

Encl.

New claims 1-44 (marked-up and clean version)
3D-structure model of CYP153A with mutations (4 pages showing different views)

NEW CLAIMS FOR AMENDMENT UNDER ARTICLE 34 PCT

PCT Application No.: PCT/US2015/036078
Applicant: REG Life Sciences, LLC
Our Ref.: PCT25752FZRPPau
Date: April 18, 2016

CLAIMS 1 – 44

1. A CYP153A-reductase hybrid fusion polypeptide variant comprising at least 90% sequence identity to SEQ ID NO: 6 and having at least one mutation at an amino acid position selected from the group consisting of 796, 141, 27, 178, 309, 407, and 415, wherein said CYP153A-reductase hybrid fusion polypeptide variant catalyzes the conversion of a fatty acid to an omega-hydroxylated fatty acid.
2. The CYP153A-reductase hybrid fusion polypeptide variant of claim 1, wherein the CYP153A-reductase hybrid fusion polypeptide variant is a hybrid cyp153A-RedRhF fusion protein variant.
3. The CYP153A-reductase hybrid fusion polypeptide variant of claim 1, wherein said mutation is selected from the group consisting of A796V, V141I, V141T, V141Q, V141G, V141M, V141L, R27L, R178N, N309R, N407A, and V415R.
4. The CYP153A-reductase hybrid fusion polypeptide variant of claim 3, wherein said mutation is selected from the group consisting of A796V, V141I, and V141T.
5. The CYP153A-reductase hybrid fusion polypeptide variant of claim 4, wherein expression of said CYP153A-reductase hybrid fusion polypeptide variant in a recombinant host cell results in a higher titer of an omega-hydroxylated fatty acid as compared to a titer of an omega-hydroxylated fatty acid produced by expression of a CYP153A-reductase hybrid fusion polypeptide of SEQ ID NO: 6.
6. The CYP153A-reductase hybrid fusion polypeptide variant of claim 3, comprising any one of SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, and SEQ ID NO: 46.
7. The CYP153A-reductase hybrid fusion polypeptide variant of claim 3, having a mutation at V141I and A231T.
8. The CYP153A-reductase hybrid fusion polypeptide variant of claim 7, comprising SEQ ID NO: 32.
9. The CYP153A-reductase hybrid fusion polypeptide variant of claim 8, wherein said CYP153A-reductase hybrid fusion polypeptide variant catalyzes the conversion of a fatty acid to an omega-hydroxylated fatty acid.

NEW CLAIMS FOR AMENDMENT UNDER ARTICLE 34 PCT

10. The CYP153A-reductase hybrid fusion polypeptide variant of claim 3, having a mutation at R27L, R82D, V141M, R178N and N407A.
11. The CYP153A-reductase hybrid fusion polypeptide variant of claim 10, comprising SEQ ID NO: 34.
12. The CYP153A-reductase hybrid fusion polypeptide variant of claim 11, wherein said CYP153A-reductase hybrid fusion polypeptide variant catalyzes the conversion of a fatty acid to an omega-hydroxylated fatty acid.
13. The CYP153A-reductase hybrid fusion polypeptide variant of claim 3, having a mutation at A796V.
14. The CYP153A-reductase hybrid fusion polypeptide variant of claim 13, comprising SEQ ID NO: 38.
15. The CYP153A-reductase hybrid fusion polypeptide variant of claim 14, wherein said CYP153A-reductase hybrid fusion polypeptide variant catalyzes the conversion of a fatty acid to an omega-hydroxylated fatty acid.
16. The CYP153A-reductase hybrid fusion polypeptide variant of claim 3, having a mutation at A796V, P666D and T516V.
17. The CYP153A-reductase hybrid fusion polypeptide variant of claim 16, comprising SEQ ID NO: 40.
18. The CYP153A-reductase hybrid fusion polypeptide variant of claim 17, wherein said CYP153A-reductase hybrid fusion polypeptide variant catalyzes the conversion of a fatty acid to an omega-hydroxylated fatty acid.
19. The CYP153A-reductase hybrid fusion polypeptide variant of claim 3, having a mutation at V141I, A231T and A796V.
20. The CYP153A-reductase hybrid fusion polypeptide variant of claim 19, comprising SEQ ID NO: 42.
21. The CYP153A-reductase hybrid fusion polypeptide variant of claim 20, wherein said CYP153A-reductase hybrid fusion polypeptide variant catalyzes the conversion of a fatty acid to an omega-hydroxylated fatty acid.
22. The CYP153A-reductase hybrid fusion polypeptide variant of claim 3, having a mutation at R27L, R82D, V141M, R178N, N407A and A796V.
23. The CYP153A-reductase hybrid fusion polypeptide variant of claim 22, comprising SEQ ID NO: 44.

NEW CLAIMS FOR AMENDMENT UNDER ARTICLE 34 PCT

24. The CYP153A-reductase hybrid fusion polypeptide variant of claim 23, wherein said CYP153A-reductase hybrid fusion polypeptide variant catalyzes the conversion of a fatty acid to an omega-hydroxylated fatty acid.
25. The CYP153A-reductase hybrid fusion polypeptide variant of claim 3, having a mutation at V141T, A231T and A796V.
26. The CYP153A-reductase hybrid fusion polypeptide variant of claim 25, comprising SEQ ID NO: 46.
27. The CYP153A-reductase hybrid fusion polypeptide variant of claim 26, wherein said CYP153A-reductase hybrid fusion polypeptide variant catalyzes the conversion of a fatty acid to an omega-hydroxylated fatty acid.
28. A recombinant host cell expressing the CYP153A-reductase hybrid fusion polypeptide variant of any one of claims 1 to 27.
29. The recombinant host cell of claim 28, further expressing a thioesterase polypeptide of EC 3.1.2.-, EC 3.1.1.5 or EC 3.1.2.14.
30. The recombinant host cell of claim 29, wherein the recombinant host cell produces a omega-hydroxylated fatty acid composition with a titer that is at least 10% greater, at least 15% greater, at least 20% greater, at least 25% greater, or at least 30% greater than the titer of an omega-hydroxylated fatty acid composition produced by a host cell expressing a corresponding CYP153A-reductase hybrid fusion polypeptide, when cultured in medium containing a carbon source.
31. The recombinant host cell of claim 30, wherein the omega-hydroxylated fatty acid is produced intracellularly.
32. The recombinant host cell of claim 31, wherein the omega-hydroxylated fatty acid is produced extracellularly.
33. A cell culture comprising the recombinant host cell of any one of claims 28 through 32.
34. The cell culture of claim 33, wherein the omega-hydroxylated fatty acid comprises one or more of a C₁₂, C₁₆ and a C_{16:1} omega-hydroxylated fatty acid.
35. The cell culture of claim 34, wherein the omega-hydroxylated fatty acid comprises an unsaturated omega-hydroxylated C_{16:1} fatty acid.
36. The cell culture of claim 34, wherein the omega-hydroxylated fatty acid comprises a saturated omega-hydroxylated C₁₂ fatty acid.
37. A method of producing an omega-hydroxylated fatty acid having an increase in titer, comprising:

NEW CLAIMS FOR AMENDMENT UNDER ARTICLE 34 PCT

- i. culturing the host cell of claim 28 with a carbon source; and
 - ii. harvesting an omega-hydroxylated fatty acid.
- 38. The method of claim 37, wherein said omega-hydroxylated fatty acid is a saturated omega-hydroxylated fatty acid.
- 39. The method of claim 37, wherein said omega-hydroxylated fatty acid is an unsaturated omega-hydroxylated fatty acid.
- 40. A recombinant microorganism for producing an omega-hydroxylated fatty acid derivative *in vivo* when grown in a fermentation broth in a presence of a carbon source from a renewable feedstock, said microorganism comprising a pathway engineered to express at least two nucleic acid sequences encoding a polypeptide comprising:
 - (i) a thioesterase of EC 3.1.2.-, EC 3.1.1.5, or EC 3.1.2.14; and
 - (ii) a CYP153A-reductase hybrid fusion polypeptide variant.
- 41. The recombinant microorganism of claim 1, wherein said CYP153A-reductase hybrid fusion polypeptide variant is a self-sufficient CYP153A-RedRhF hybrid fusion protein variant.
- 42. The recombinant microorganism of claim 1, wherein said CYP153A-reductase hybrid fusion polypeptide variant has at least 90% sequence identity to any one of SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44 and SEQ ID NO: 46.
- 43. A CYP153A-reductase hybrid fusion polypeptide variant comprising at least 90% sequence identity to SEQ ID NO: 38 and having at least one mutation at an amino acid position selected from the group consisting of 747, 12, 327, 14, 61, 28, 13, 771, 119, 10, 11, 28, 745, 9, 770, 413, 784, 749, 231, 233, 757 and 703, wherein said CYP153A-reductase hybrid fusion polypeptide variant catalyzes the conversion of a fatty acid to an omega-hydroxylated fatty acid.
- 44. The CYP153A-reductase hybrid fusion polypeptide variant of claim 43, wherein said mutation is selected from the group consisting of D747N, Q12W, Q12T, Q12R, P327D, R14F, N61L, Q28M, S13K, V771F, K119R, D10Y, I11L, Q28T, P745R, D9N, D9K, T770G, Y413R, M784I, E749L, A231Y, S233L, E757A, and L703G.