Summary

Provided in certain aspects is a genetically modified yeast comprising (i) an active, modified endogenous acyl-coA oxidase polypeptide or an active, modified endogenous acyl-coA dehydrogenase polypeptide; (ii) a genetic modification that alters an enoyl co-A isomerase activity; and (iii) a genetic modification that alters a dienoyl CoA reductase (DCR) activity, wherein the yeast is capable of producing a diacid from a feedstock comprising one or more components from a vegetable oil. Also provided in some aspects is a method for using such a genetically modified yeast to produce a diacid from a feedstock comprising one or more components from a vegetable oil.

Also provided in certain aspects is a method for producing a diacid, comprising: (a) contacting a genetically modified yeast of any of embodiments A1 to A44, B1 to B33, C1 to C15 and G1 to G65 with a feedstock comprising an unsaturated vegetable fatty acid capable of being converted by the yeast to a diacid; (b) culturing the yeast under conditions in which diacid containing one or more unsaturations is produced from the feedstock; and (c) hydrogenating and removing one or more of the unsaturations.
Certain embodiments are described further in the following description, examples, claims and drawings.

Also provided in certain aspects is a genetically modified Yarrowia lipoytica yeast comprising (i) an acyl-CoA oxidase polypeptide, wherein the acyl-CoA oxidase polypeptide comprises a genetic modification that alters the enzyme activity of native acyl-CoA oxidase polypeptide; or an acyl-CoA dehydrogenase polypeptide, wherein the acyl-CoA dehydrogenase polypeptide comprises a genetic modification that alters the enzyme activity of native acyl-CoA dehydrogenase polypeptide; (ii) an enoyl CoA isomerase polypeptide, wherein the enoyl CoA isomerase polypeptide comprises a genetic modification that alters the enzyme activity of a native enoyl CoA isomerase polypeptide; and (iii) a dieneoyl CoA reductase (DCR) polypeptide, wherein the DCR polypeptide comprises a genetic modification that alters the enzyme activity of a native polypeptide, wherein the yeast is capable of producing a diacid from a feedstock comprising one or more components from a vegetable oil; and wherein the genetic modification is performed by:

(i) adding a heterologous polynucleotide that encodes said polypeptide;

(ii) adding a regulatory sequence that regulates the expression of said polypeptide;
(iii) disrupting the polynucleotide that encodes said polypeptide;
(iv) deleting the regulatory sequence that regulates the expression of said polypeptide;
(v) deleting the coding sequence that encodes said polypeptide; or
(vi) any combination thereof.

Also provided in certain aspects is a method for producing a diacid, comprising:

(1) contacting a genetically modified yeast as described herein with a feedstock comprising one or more components from a feedstock containing at least one unsaturated vegetable fatty acid capable of being converted by the yeast to a diacid;
(2) culturing the yeast under conditions in which the diacid is produced from the feedstock; and
(3) hydrogenating the diacid to remove any unsaturation in the carbon chain.

Also provided in certain aspects is a method for producing a diacid, comprising:

(a) contacting a genetically modified yeast as described herein with a feedstock comprising a vegetable oil; and
(b) culturing the yeast under conditions in which diacid is produced from the feedstock.

Also provided in certain aspects is a method for producing a diacid, comprising:

(a) contacting a genetically modified yeast as described herein with a feedstock comprising an unsaturated vegetable fatty acid capable of being converted by the yeast to a diacid;
(b) culturing the yeast under conditions in which diacid containing one or more unsaturations is produced from the feedstock; and
(c) hydrogenating and removing one or more of the unsaturations.

Brief Description of the Drawings

The drawings illustrate embodiments of the technology and are not limiting. For clarity and ease of illustration, the drawings are not made to scale and, in some instances, various aspects may be shown exaggerated or enlarged to facilitate an understanding of particular embodiments.

FIG. 1 is a schematic representation of the conversion of decane to sebacic acid in a beta-oxidation blocked microorganism. Capric acid is formed as an intermediate during omega oxidation.

FIG. 2 is a schematic representation of the conversion of dodecane to dodecanedioic acid in a beta-oxidation blocked microorganism. Lauric acid is formed as an intermediate during omega oxidation.

What is claimed is:

1. A genetically modified Yarrowia lipoytica yeast comprising (i) an acyl-CoA oxidase polypeptide, wherein the acyl-CoA oxidase polypeptide comprises a genetic modification that alters the enzyme activity of native acyl-CoA oxidase polypeptide; or an acyl-CoA dehydrogenase polypeptide, wherein the acyl-CoA dehydrogenase polypeptide comprises a genetic modification that alters the enzyme activity of native acyl-CoA dehydrogenase polypeptide; (ii) an enoyl CoA isomerase polypeptide, wherein the enoyl CoA isomerase polypeptide comprises a genetic modification that alters the enzyme activity of ~~a~~ native enoyl CoA isomerase polypeptide; and (iii) a dieneoyl CoA reductase (DCR) polypeptide, wherein the DCR polypeptide comprises a genetic modification that alters the enzyme activity of ~~a~~ native polypeptide, wherein the yeast is capable of producing a diacid from a feedstock comprising one or more components from a vegetable oil; and wherein the genetic modification is performed by:
   (i) adding a heterologous polynucleotide that encodes said polypeptide;
   (ii) adding a regulatory sequence that regulates the expression of said polypeptide;
   (iii) disrupting the polynucleotide that encodes said polypeptide;
   (iv) deleting the regulatory sequence that regulates the expression of said polypeptide;
   (v) deleting the coding sequence that encodes said polypeptide; or
   (vi) any combination thereof.

2. A method for producing a diacid, comprising:
   (1) contacting a genetically modified yeast of claim 1 with a feedstock comprising one or more components from a feedstock containing at least one unsaturated vegetable fatty acid capable of being converted by the yeast to a diacid;
   (2) culturing the yeast under conditions in which the diacid is produced from the feedstock; and
   (3) hydrogenating the diacid to remove any unsaturation in the carbon chain.

3. The method of claim 2, wherein the unsaturated vegetable fatty acid is linoleic acid or linolenic acid.

4. The method of claim 3, wherein the diacid is dodecanedioic acid or sebacic acid.

5. The genetically modified yeast of claim 1, comprising one or more genetic modifications that reduce the activity of one or more native endogenous acyl-CoA oxidase polypeptides.

6. The genetically modified yeast of claim 5, comprising genetic modifications that reduce the activity of all native endogenous acyl-CoA oxidase polypeptides.

7. The genetically modified yeast of claim 5 or 6, wherein the genetic modifications partially block beta oxidation activity.

8. The genetically modified yeast of any one of claims 1 or 5-7, wherein the diacid is a C4 to C24 diacid.

9. The genetically modified yeast of claim 8, wherein the diacid is a C10, C12, C14, C16, C18 or C20 diacid.

10. The genetically modified yeast of claim 9, wherein the diacid is a C10 diacid.

11. The genetically modified yeast of claim 9, wherein the diacid is a C12 diacid.

12. The genetically modified yeast of claim 9, wherein the diacid is a C18 diacid.

13. The genetically modified yeast of any one of claims 8-12, wherein the diacid contains no unsaturation.

14. The genetically modified yeast of any one of claims 8-12, wherein the diacid contains one or more unsaturations.

15. The genetically modified yeast of any one of claims 8-14, wherein the diacid is the predominant diacid in a mixture of diacids.

16. The genetically modified yeast of any one of claims 1 or 5-15, wherein the feedstock comprises a substantially pure oil.

17. The genetically modified yeast of any one of claims 1 or 5-16, wherein the feedstock comprises a plurality of fatty acids.

18. The genetically modified yeast of claim 17, wherein the feedstock comprises a soapstock.

19. The genetically modified yeast of claim 17, wherein the feedstock comprises a fatty acid distillate.

20. The genetically modified yeast of any one of claims 1 or 5-19, wherein the vegetable oil is from a plant selected from the group consisting of: palm, palm kernel, coconut, soy, safflower, canola, palm, palm kernel or combination thereof.

21. A method for producing a desired diacid, comprising:
(i) contacting a genetically modified yeast of any one of claims 1 or 5-20 with a feedstock comprising one or more components from a vegetable oil capable of being converted by the yeast to a diacid; and
(ii) culturing the yeast under conditions in which the desired diacid is produced from the feedstock.

22. The method of claim 21, wherein the desired diacid is a C4 to C24 diacid.

23. The method of any one of claims 21-22, wherein the desired diacid contains no unsaturation.

24. The method of any one of claims 21-22, wherein the desired diacid contains one or more unsaturations.

25. The method of any one of claims 21-24, wherein the desired diacid is the predominant diacid in a mixture of diacids.

26. The method of any one of claims 21-25, wherein the feedstock comprises a substantially pure oil.

27. The method of any one of claims 21-25, wherein the feedstock comprises a plurality of fatty acids.

28. The method of claim 27, wherein the feedstock comprises a soapstock.

29. The method of claim 27, wherein the feedstock comprises a fatty acid distillate.

30. The method of any one of claims 21-29, wherein the vegetable oil is from a plant selected from the group consisting of: palm, palm kernel, coconut, soy, safflower, canola, palm, palm kernel or combination thereof.

31. A genetically modified yeast comprising (i) an active, modified endogenous acyl-coA oxidase polypeptide or an active, modified endogenous acyl-coA dehydrogenase polypeptide; (ii) a genetic modification that alters an enoyl co-A isomerase activity; and (iii) a genetic modification that alters a dieneoyl CoA reductase (DCR) activity, wherein the yeast is capable of producing a diacid from a feedstock comprising one or more components from a vegetable oil.

32. The genetically modified yeast of claim 31, wherein the feedstock comprises at least one long chain unsaturated fatty acid.

33. The genetically modified yeast of claim 32, wherein the long chain unsaturated fatty acid is linoleic acid or linolenic acid.

34. The genetically modified yeast of any one of claims 31-33, which comprises a genetic modification that reduces an enoyl CoA isomerase activity.

35. The genetically modified yeast of claim 34, which comprises a disruption, deletion or knockout of (i) a polynucleotide that encodes an enoyl CoA isomerase polypeptide or (ii) a promoter operably linked to a polynucleotide that encodes an enoyl CoA isomerase polypeptide, whereby the enoyl CoA isomerase activity is reduced or removed.

36. The genetically modified yeast of claim 35, wherein the genetic modification disrupts a polynucleotide that encodes an enoyl CoA isomerase polypeptide.

37. The genetically modified yeast of any one of claims 31-36, which comprises a genetic modification that increases an enoyl CoA isomerase activity.

38. The genetically modified yeast of claim 37, which comprises (i) an increased copy number of a polynucleotide that encodes an enoyl CoA isomerase polypeptide or (ii) a promoter inserted and operably linked to a polynucleotide that encodes an enoyl CoA isomerase polypeptide.

39. The genetically modified yeast of any one of claims 31-38, wherein the enoyl CoA isomerase activity is provided by a polypeptide comprising the amino acid sequence of SEQ ID NO: 3675, 3677, 3800, 3802, 3803 or 3805.

40. The genetically modified yeast of any one of claims 31-38, wherein enoyl CoA isomerase activity is provided by a polypeptide present in a Yarrowia yeast.

41. The genetically modified yeast of claim 40, wherein the Yarrowia yeast is a Y. lipolytica yeast.

42. The genetically modified yeast of any one of claims 31-38, wherein enoyl CoA isomerase activity is provided by a polypeptide comprising the amino acid sequence of SEQ ID NO: 3804 or 3806.

43. The genetically modified yeast of any one of claims 31-42, which comprises a genetic modification that reduces a dieneoyl CoA reductase activity.

44. The genetically modified yeast of claim 43, which comprises a disruption, deletion or knockout of (i) a polynucleotide that encodes a dieneoyl CoA reductase polypeptide or (ii) a promoter operably linked to a polynucleotide that encodes a dieneoyl CoA reductase polypeptide, whereby the dieneoyl CoA reductase activity is reduced or removed.

45. The genetically modified yeast of claim 44, wherein the genetic modification disrupts a polynucleotide that encodes a dieneoyl CoA reductase polypeptide.

46. The genetically modified yeast of any one of claims 31-42, which comprises a genetic modification that increases a dieneoyl CoA reductase activity.

47. The genetically modified yeast of claim 46, which comprises (i) an increased copy number of a polynucleotide that encodes a dieneoyl CoA reductase polypeptide or (ii) a promoter inserted and operably linked to a polynucleotide that encodes a dieneoyl CoA reductase polypeptide.

48. The genetically modified yeast of any one of claims 31-47, wherein the dieneoyl CoA reductase activity is provided by a polypeptide comprising the amino acid sequence of SEQ ID NO: 3789, 3791, 3792 or 3793.

49. The genetically modified yeast of any one of claims 31-48, wherein the dieneoyl CoA reductase activity is provided by a polypeptide present in a Yarrowia yeast.

50. The genetically modified yeast of claim 49, wherein the Yarrowia yeast is a Y. lipolytica yeast.

51. The genetically modified yeast of any one of claims 31-48, wherein the dieneoyl CoA reductase activity is provided by a polypeptide comprising the amino acid sequence of SEQ ID NO: 3794 or 3795.

52. The genetically modified yeast of any one of claims 31-51, wherein the endogenous acyl-CoA oxidase polypeptide is a POX4 polypeptide.

53. The genetically modified yeast of claim 52, wherein the POX4 polypeptide comprises a modified amino acid sequence of SEQ ID NO: 30.

54. The genetically modified yeast of claim 52 or 53, wherein the POX4 polypeptide comprises an amino acid modification at one or more amino acid positions selected from the group consisting of: 88, 90, 96, 98, 99, 100, 102, 103, 302, 309, 310, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504 and 505.

55. The genetically modified yeast of any one of claims 31-51, wherein the endogenous acyl-CoA oxidase polypeptide is a POX5 polypeptide.

56. The genetically modified yeast of claim 55, wherein the POX5 polypeptide comprises a modified amino acid sequence of SEQ ID NO: 32.

57. The genetically modified yeast of claim 55, wherein the POX5 polypeptide comprises an amino acid modification at one or more amino acid positions selected from the group consisting of: 81, 82, 83, 84, 85, 86, 88, 93, 94, 95, 96, 98, 102, 284, 287, 290, 291, 292, 294, 295, 428, 429, 436, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462 and 463.

58. The genetically modified yeast of any one of claims 31-57, wherein the acyl-CoA dehydrogenase polypeptide is selected from the group consisting of: ACAD, VLCAD, LCAD, MCAD and SCAD polypeptides.

59. The genetically modified yeast of claim 58, wherein the acyl-CoA dehydrogenase polypeptide comprises a modified amino acid sequence of SEQ ID NO: 3685.

60. The genetically modified yeast of any one of claims 31-59, wherein the modified endogenous acyl-CoA oxidase polypeptide comprises an amino acid modification in the N-terminal loop.

61. The genetically modified yeast of any one of claims 31-60, wherein the modified endogenous acyl-CoA oxidase polypeptide comprises an amino acid modification in the D alpha helix.

62. The genetically modified yeast of any one of claims 31-61, wherein the modified endogenous acyl-CoA oxidase polypeptide comprises an amino acid modification in the loop between the D alpha helix and the E' alpha helix.

63. The genetically modified yeast of any one of claims 31-62, wherein the modified endogenous acyl-CoA oxidase polypeptide comprises an amino acid modification to an amino acid in effective contact with carbons 6 to 9 in a feedstock component.

64. The genetically modified yeast of any one of claims 31-63, wherein the modified endogenous acyl-CoA oxidase polypeptide comprises an amino acid modification to an amino acid in effective contact with carbons 10 to 12 in a feedstock component.

65. The genetically modified yeast of any one of claims 31-64, wherein the modified endogenous acyl-CoA oxidase polypeptide comprises an amino acid modification in the L alpha helix.

66. The genetically modified yeast of any one of claims 31-65, wherein the modified endogenous acyl-CoA oxidase polypeptide comprises an amino acid modification in the loop C-terminal to the L alpha helix.

67. The genetically modified yeast of any one of claims 31-66, wherein the modified endogenous acyl-CoA oxidase polypeptide comprises an amino acid modification in the loop between the L alpha helix and the M alpha helix.

68. A method for producing a diacid, comprising:
    (a) contacting a genetically modified yeast of any one of claims 31-67 with a feedstock comprising a vegetable oil; and
    (b) culturing the yeast under conditions in which diacid is produced from the feedstock.

69. The method of claim 68, comprising purifying the diacid from the yeast.

70. A method for producing a diacid, comprising:
    (a) contacting a genetically modified yeast of any one of claims 1 or 5-20 and 31-69 with a feedstock comprising an unsaturated vegetable fatty acid capable of being converted by the yeast to a diacid;
    (b) culturing the yeast under conditions in which diacid containing one or more unsaturations is produced from the feedstock; and
    (c) hydrogenating and removing one or more of the unsaturations.

71. The method of claim 70, wherein the unsaturated vegetable fatty acid is linoleic acid or linolenic acid.