Summary

In one aspect, there is provided a genetically modified yeast, comprising:
a genetic modification that blocks beta oxidation activity;

a genetic modification that increase an activity selected from the group consisting of: monooxygenase activity, monooxygenase reductase activity, thioesterase activity, acyltransferase activity, isocitrate dehydrogenase activity, glyceraldehyde-3-phosphate dehydrogenase activity, glucose-6-phosphate dehydrogenase activity, acyl-coA oxidase activity, fatty alcohol oxidase activity, acyl-CoA hydrolase activity, alcohol dehydrogenase activity, peroxisomal biogenesis factor activity, fatty aldehyde dehydrogenase activity, CTF, UTR, FAT1; and

a genetic modification that decreases MIG1 activity, wherein the genetically modified yeast produces a higher yield of a diacid as compared to corresponding yeast not comprising said genetic modification when cultured with a feedstock comprising at least one fatty acid or an ester thereof.

In one aspect, there is provided a method for producing a desired diacid, comprising:

contacting a genetically modified yeast as described herein with a feedstock comprising at least one fatty acid or an ester thereof capable of being converted by the yeast to a diacid; and

culturing the yeast under conditions in which the desired diacid is produced from the feedstock; wherein the desired diacid is a C10, C12, C14, C16, C18 or C20 diacid.

In one aspect, there is provided a method for producing a desired diacid by a yeast from a feedstock toxic to the yeast, comprising:

(a) contacting a genetically modified yeast in culture with a feedstock not substantially toxic to the yeast, thereby performing an induction; and

(b) contacting the yeast after the induction in (a) with a feedstock toxic to the yeast,

whereby the desired diacid is produced by the yeast from the feedstock toxic to the yeast in an amount greater than the amount of the diacid produced from the feedstock toxic to the yeast when the induction is not performed.

In one aspect, there is provided a diacid produced by the method as described herein.

What is claimed is:

1. A genetically modified yeast, comprising:

a genetic modification that blocks beta oxidation activity;

a genetic modification that increase an activity selected from the group consisting of: monooxygenase activity, monooxygenase reductase activity, thioesterase activity, acyltransferase activity, isocitrate dehydrogenase activity, glyceraldehyde-3-phosphate dehydrogenase activity, glucose-6-phosphate dehydrogenase activity, acyl-coA oxidase activity, fatty alcohol oxidase activity, acyl-CoA hydrolase activity, alcohol dehydrogenase activity, peroxisomal biogenesis factor activity, fatty aldehyde dehydrogenase activity, CTF, UTR, FAT1; and

a genetic modification that decreases MIG1 activity, wherein the genetically modified yeast produces a higher yield of a diacid as compared to corresponding yeast not comprising said genetic modification when cultured with a feedstock comprising at least one fatty acid or an ester thereof.

2. The genetically modified yeast of claim 1, comprising a genetic modification that increases one or more monooxygenase activities selected from a CYP52A12 monooxygenase activity, CYP52A13 monooxygenase activity, CYP52A14 monooxygenase activity, CYP52A15 monooxygenase activity, CYP52A16 monooxygenase activity, CYP52A17 monooxygenase activity, CYP52A18 monooxygenase activity, CYP52A19 monooxygenase activity, CYP52A20 monooxygenase activity, CYP52D2 monooxygenase activity and BM3 monooxygenase activity.

3. The genetically modified yeast according to one of claims 1-2, comprising a genetic modification that increases one or more monooxygenase reductase activities selected from the group consisting of: CPRA monooxygenase reductase activity, CPRB monooxygenase reductase activity and CPR750 monooxygenase reductase activity.

4. The genetically modified yeast according to one of claims 1-3 comprising a genetic modification that increases IDP2 isocitrate dehydrogenase activity.

5. The genetically modified yeast according to one of claims 1-4 comprising a genetic modification that increases a GDP1 glyceraldehyde-3-phosphate dehydrogenase activity.

6. The genetically modified yeast according to one of claims 1-5, comprising a genetic modification that increases a glucose-6-phosphate dehydrogenase activity selected from the group consisting of: a ZWF1 glucose-6-phosphate dehydrogenase activity and ZWF2 glucose-6-phosphate dehydrogenase activity.

7. The genetically modified yeast according to one of claims 1-6, comprising a genetic modification that increases one or more fatty alcohol oxidase activities selected from the group consisting of: FAO1 fatty alcohol oxidase activity, FAO2A fatty alcohol oxidase activity, FAO2B fatty alcohol oxidase activity, FAO13 fatty alcohol oxidase activity, FAO17 fatty alcohol oxidase activity, FAO18 fatty alcohol oxidase activity and FAO20 fatty alcohol oxidase activity.

8. The genetically modified yeast according to one of claims 1-7, comprising a genetic modification that increases one or more alcohol dehydrogenase activities selected from the group consisting of: ADH1 alcohol dehydrogenase activity, ADH2 alcohol dehydrogenase activity, ADH3 alcohol dehydrogenase activity, ADH4 alcohol dehydrogenase activity, ADH5 alcohol dehydrogenase activity, ADH7 alcohol dehydrogenase activity, ADH8 alcohol dehydrogenase activity and SFA alcohol dehydrogenase activity.

9. The genetically modified yeast according to one of claims 1-8, comprising a genetic modification that increases one or more acyl-CoA hydrolase activities selected from the group consisting of: ACH-A acyl-CoA hydrolase activity and ACH-B acyl-CoA hydrolase activity.

10. The genetically modified yeast according to one of claims 1-9, comprising a genetic modification that increases one or more acyltransferase activities selected from the group consisting of: acyl-CoA sterol acyltransferase activity, diacylglycerol acyltransferase activity and phospholipid:diacylglycerol acyltransferase activity.

11. The genetically modified yeast according to claim 10, wherein the one or more acyltransferase activities are selected from the group consisting of: ARE1 acyl-CoA sterol acyltransferase activity, ARE2 acyl-CoA sterol acyltransferase activity, DGA1 diacylglycerol acyltransferase activity, and LRO1 phospholipid:diacylglycerol acyltransferase activity.

12. The genetically modified yeast according to any one of claims 1 to 11, comprising a genetic modification that increases an acyl-coA thioesterase activity.

13. The genetically modified yeast according to claim 12, wherein the acyl-coA thioesterase activity is a TESA acyl-coA thioesterase activity.

14. The genetically modified yeast according to any one of claims 1 to 13, comprising a genetic modification that increases a PEX11 peroxisomal biogenesis factor activity.

15. The genetically modified yeast according to any one of claims 1 to 14, comprising a genetic modification that increases one or more fatty aldehyde dehydrogenase activities selected from the group consisting of: HFD1 fatty aldehyde dehydrogenase activity and HFD2 fatty aldehyde dehydrogenase activity.

16. The genetically modified yeast according to any one of claims 1 to 15, comprising a genetic modification that increases a POX5 acyl-coA oxidase activity.

17. The genetically modified yeast according to any one of claims 1 to 13, comprising a genetic modification that increases a monooxygenase activity and a monooxygenase reductase activity.

18. The genetically modified yeast according to claim 17, wherein the genetic modification increases a CYP52A19 monooxygenase activity and a CPRB monooxygenase reductase activity.

19. The genetically modified yeast according to claim 17, wherein the genetic modification increases a CYP52A14 monooxygenase activity and a CPRB monooxygenase reductase activity.

20. The genetically modified yeast according to any one of claims 1-19, comprising one or more genetic modifications resulting in an increase in a monooxygenase activity, a monooxygenase reductase activity, and a isocitrate dehydrogenase activity.

21. The genetically modified yeast according to claim 20, wherein one or more genetic modifications increase a CYP52A19 monooxygenase activity, a CPRB monooxygenase reductase activity, and a IDP2 isocitrate dehydrogenase activity.

22. The genetically modified yeast according to any one of claims 1 to 21, comprising a genetic modification that increases a monooxygenase activity, a monooxygenase reductase activity, and a glucose-6-phosphate dehydrogenase activity.

23. The genetically modified yeast according to claim 22, comprising a genetic modification that increases a CYP52A19 monooxygenase activity, a CPRB monooxygenase reductase activity, and a ZWF1 glucose-6-phosphate dehydrogenase activity.

24. The genetically modified yeast according to any one of claims 1 to 23, wherein said monooxygenase activity is by a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 60 and 61.

25. The genetically modified yeast according to claim 24, wherein the polypeptide is encoded by a polynucleotide selected from the group consisting of: SEQ ID NOs: 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24.

26. The genetically modified yeast according to any one of claims 1-25, wherein said monooxygenase reductase activity is by a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs: 28, 63, 64 and 65.

27. The genetically modified yeast according to claim 26, wherein the polypeptide is encoded by a polynucleotide selected from the group consisting of: SEQ ID NOs: 24, 25, 26 and 27.

28. The genetically modified yeast according to any one of claims 1-27, wherein a thioesterase activity is by a polypeptide comprising an amino acid sequence of SEQ ID NO: 38.

29. The genetically modified yeast according to claim 28, wherein the polypeptide is encoded by a polynucleotide of SEQ ID NO: 37.

30. The genetically modified yeast according to any one of claims 1-29, wherein an acyltransferase activity is by a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs: 44, 46, 48 and 50.

31. The genetically modified yeast according to claim 30, wherein the polypeptide is encoded by a polynucleotide selected from the group consisting of: SEQ ID NOs: 43, 45, 47 and 49.

32. The genetically modified yeast according to any one of claims 1 to 31, wherein an isocitrate dehydrogenase activity is by a polypeptide comprising an amino acid sequence of SEQ ID NO: 67, 69 or 100.

33. The genetically modified yeast according to claim 32, wherein the polypeptide is encoded by a polynucleotide of SEQ ID NO: 68, 70 or 99.

34. The genetically modified yeast according to any one of claims 1-34, wherein a glyceraldehyde-3-phosphate dehydrogenase activity is by a polypeptide comprising an amino acid sequence of SEQ ID NO: 72.

35. The genetically modified yeast according to claim 34, wherein the polypeptide is encoded by a polynucleotide of SEQ ID NO: 71.

36. The genetically modified yeast according to any one of claims 1-35, wherein a glucose-6-phosphate dehydrogenase activity is by a polypeptide comprising an amino acid sequence of SEQ ID NO: 74, 76 or 157.

37. The genetically modified yeast according to claim 36, wherein the polypeptide is encoded by a polynucleotide of SEQ ID NO: 73 or 75.

38. The genetically modified yeast according to any one of claims 1-37, wherein an acyl-coA oxidase activity is by a polypeptide comprising an amino acid sequence of SEQ ID NO: 32.

39. The genetically modified yeast according to claim 38, wherein the polypeptide is encoded by a polynucleotide of SEQ ID NO: 31.

40. The genetically modified yeast according to any one of claims 1-39, wherein a fatty alcohol oxidase activity is by a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs: 3, 5, 7, 9, 11, 13, 132 and 134.

41. The genetically modified yeast according to claim 40, wherein the polypeptide is encoded by a polynucleotide selected from the group consisting of: SEQ ID NOs: 2, 4, 6, 8, 10, 12, 131 and 133.

42. The genetically modified yeast according to any one of claims 1-41, wherein an acyl-CoA hydrolase activity is by a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs: 34 and 36.

43. The genetically modified yeast according to claim 42, wherein the polypeptide is encoded by a polynucleotide selected from the group consisting of: SEQ ID NOs: 33 and 35.

44. The genetically modified yeast according to any one of claims 1-43, wherein an alcohol dehydrogenase activity is by a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs: 129, 113, 115, 117, 119, 121, 123, 125, 127, 152 and 154.

45. The genetically modified yeast according to claim 44, wherein the polypeptide is encoded by a polynucleotide selected from the group consisting of: SEQ ID NOs: 130, 114, 116, 118, 120, 122, 124, 126, 128, 153 and 155.

46. The genetically modified yeast according to any one of claims 1-45, wherein a peroxisomal biogenesis factor activity is by a polypeptide comprising an amino acid sequence of SEQ ID NO: 96.

47. The genetically modified yeast according to claim 46, wherein the polypeptide is encoded by a polynucleotide of SEQ ID NO: 95.

48. The genetically modified yeast according to any one of claims 1-47, wherein a fatty aldehyde dehydrogenase activity is by a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs: 139 and 141.

49. The genetically modified yeast according to claim 48, wherein the polypeptide is encoded by a polynucleotide selected from the group consisting of: SEQ ID NOs: 140 and 142.

50. The genetically modified yeast according to any one of claims 1-49, comprising a genetic modification that decreases an acyl-coA synthetase activity.

51. The genetically modified yeast according to claim 50, wherein the genetic modification decreases one or more acyl-coA synthetase activities selected from the group consisting of: ACS1 acyl-coA synthetase activity and FAT1 long-chain acyl-CoA synthetase activity.

52. The genetically modified yeast according to any one of claims 50-51, wherein a genetic modification disrupts a nucleic acid that encodes a polypeptide having the acyl-coA synthetase activity.

53. The genetically modified yeast according to any one of claims 50-52, wherein the acyl-coA synthetase activity is by a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs: 40, 42, 80, 82, 84, 90, 158 and 159.

54. The genetically modified yeast according to claim 53, wherein the polypeptide is encoded by a polynucleotide selected from the group consisting of: SEQ ID NOs: 39, 41, 79, 81, 83 and 89.

55. The genetically modified yeast according to any one of claims 1-54, which is a Candida spp. yeast.

56. The genetically modified yeast according to claim 55, wherein the Candida spp. yeast is selected from the group consisting of: C. tropicalis and C. viswanathii.

57. The genetically modified yeast according to claim 56, wherein the Candida spp. yeast is a genetically modified ATCC20336 yeast.

58. The genetically modified yeast according to any one of claims 1-54, which is selected from the group consisting of: a Yarrowia spp. yeast, Pichia spp. yeast, Saccharomyces spp. yeast and Kluyveromyces spp. yeast.

59. The genetically modified yeast according to claim 58, which is selected from the group consisting of: Y. lipolytica, P. pastoris, P. membranifaciens, P. kluyveri, P. guilliermondii, P. heedii, P. subpelliculosa, S. cerevisiae, S. bayanus, S. pastorianus, S. carlsbergensis, K. lactis and K. marxianus.

60. The genetically modified yeast according to any one of claims 1-59, wherein said feedstock comprises at least one component obtained from a vegetable oil.

61. The genetically modified yeast according to claim 60, wherein the desired diacid is a C4 to C24 diacid.

62. The genetically modified yeast according to claim 61, wherein the desired diacid is a C10, C12, C14, C16, C18 or C20 diacid.

63. The genetically modified yeast according to claim 62, wherein the desired diacid is a C10 diacid.

64. The genetically modified yeast according to claim 62, wherein the desired diacid is a C12 diacid.

64. The genetically modified yeast according to claim 62, wherein the desired diacid is a C18 diacid.

65. The genetically modified yeast according to any one of claims 60-64, wherein the desired diacid contains no unsaturation.

66. The genetically modified yeast according to any one of claims 60-64, wherein the desired diacid contains one or more unsaturations.

67. The genetically modified yeast according to any one of claims 60-66, wherein the desired diacid is the predominant diacid in a mixture of diacids.

68. The genetically modified yeast according to any one of claims 60-67, wherein the feedstock comprises a pure oil.

69. The genetically modified yeast according to any one of claims 60-68, wherein the feedstock comprises a plurality of fatty acids.

70. The genetically modified yeast according to claim 69, wherein the feedstock comprises a soapstock.

71. The genetically modified yeast according to claim 69, wherein the feedstock comprises a fatty acid distillate.

72. The genetically modified yeast according to any one of claims 60-71, wherein the vegetable oil is from a plant selected from the group consisting of: palm, palm kernel, coconut, soy, safflower, canola or combination thereof.

73. The genetically modified yeast according to any one of claims 1-72, wherein a genetic modification that increases an activity comprises incorporating in the yeast multiple copies of a polynucleotide that encodes a polypeptide having the activity.

74. The genetically modified yeast according to any one of claims 1-73, wherein a genetic modification that increases an activity comprises incorporating in the yeast a promoter in operable linkage with a polynucleotide that encodes a polypeptide having the activity.

75. The genetically modified yeast according to claim 74, wherein the promoter is native to the yeast.

76. The genetically modified yeast according to any one of claims 74-75, wherein the promoter is selected from the group consisting of: a POX4 promoter, PEX11 promoter, TEF1 promoter, PGK promoter and FAO1 promoter.

77. The genetically modified yeast according to claim 76, wherein the promoter comprises a polynucleotide selected from the group consisting of: SEQ ID NOs: 162, 165, 166, 167 and 169.

78. A method for producing a desired diacid, comprising:

contacting a genetically modified yeast of any one of claims 1-77 with a feedstock comprising at least one fatty acid or an ester thereof capable of being converted by the yeast to a diacid; and

culturing the yeast under conditions in which the desired diacid is produced from the feedstock; wherein the desired diacid is a C10, C12, C14, C16, C18 or C20 diacid.

79. The method of claim 78, wherein the feedstock comprises one or more components from a vegetable oil.

80. The method of any one of claims 78-79, wherein the desired diacid is a C4 to C24 diacid.

81. The method of claim 80, wherein the desired diacid is a C10 diacid.

82. The method of claim 80, wherein the desired diacid is a C12 diacid.

83. The method of claim 80, wherein the desired diacid is a C18 diacid.

84. The method of any one of claims 78-83, wherein the desired diacid contains no unsaturation.

85. The method of any one of claims 78-83, wherein the desired diacid contains one or more unsaturations.

86. The method of any one of claims 78-85, wherein the desired diacid is the predominant diacid in a mixture of diacids.

87. The method of any one of claims 78-86, wherein the feedstock comprises a substantially pure oil.

88. The method of any one of claims 78-86, wherein the feedstock comprises a plurality of fatty acids.

89. The method of claim 88, wherein the feedstock comprises a soapstock.

90. The method of claim 88, wherein the feedstock comprises a fatty acid distillate.

91. The method of any one of claims 78-90, wherein the vegetable oil is from a plant selected from the group consisting of: palm, palm kernel, coconut, soy, safflower, canola or combination thereof.

92. The method of any one of claims 78-84 wherein the feedstock comprises a fatty acid methyl ester.

93. The method of claim 92 wherein the fatty acid methyl ester is methyl laurate and the diacid comprises dodecanedioic acid.

94. The method of any one of claims 78-84 wherein the feedstock comprises a fatty acid ethyl ester and the diacid comprises dodecanedioic acid.

95. The method of any one of claims 78-84 wherein the feedstock comprises lauric acid and the diacid comprises dodecanedioic acid.

96. The method of any one of claims 78-84 wherein the feedstock comprises ethyl caprate and the diacid comprises sebacic acid.