

CLAIMS

WHAT IS CLAIMED IS:

1. A recombinant lipase comprising an amino acid sequence comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 2.

2. The recombinant lipase of Claim 1, wherein said recombinant lipase comprises a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 94, 350, 442, 540, 646, 758, and/or 868.

3. The recombinant lipase of Claim 1, wherein said recombinant lipase comprises a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 2, and wherein said polypeptide sequence comprises at least one substitution or substitution set at one or more positions selected from 2, 3, 4, 22, 27/46/97/136/149/385, 27/46/385, 27/70/136/231/385, 27/136/323/385, 27/149, 27/149/385, 27/385, 34, 38, 41, 44, 46/70, 46/149/183/231/385, 48, 70, 73, 73/305, 82, 82/94/101/194/199, 82/94/199, 83, 85, 87, 89, 92, 94, 96, 97/149/385, 135, 136/385, 140, 141, 142, 144, 146, 149, 149/231/385, 151, 174, 175, 178, 181, 183/385, 189, 194, 194/199, 195, 195/231/385, 199, 199/213, 210, 212, 213, 213/330, 216, 218, 219, 226, 231/385, 238, 247, 250, 270, 274, 281, 292, 296, 300, 308, 330, 338, 379, and 385, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2.

4. The recombinant lipase of Claim 1, wherein said recombinant lipase comprises a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 94, and wherein said polypeptide sequence comprises at least one substitution or substitution set at one or more positions selected from 3/4/96/296/300, 3/292/296, 4, 4/11/149/292/296/300, 4/96/149/231/296/300, 4/96/149/292/296/300, 4/96/219/296, 4/96/231/296/300, 4/96/292/296/300, 4/149/174, 4/174/219/292/296, 4/231/296/300, 27, 27/34/82, 27/73/82/218, 27/89, 27/89/178, 27/89/218, 27/218, 34/73/218, 34/82, 34/218, 73/82, 73/82/183, 94/146/175, 96/149/174/292/296, 96/149/231/292/296, 96/149/292/296, 96/174/219/231/292/296, 96/231/296, 146/175/189/281, 149/174/292/296, 149/174/300, 149/219/231/292/296/300, 149/231/292/296, 149/231/292/296/300, 149/296, 149/296/300, 174/231, 174/296, 218, 231, 231/292/296, 231/292/296/300, 231/296, 231/300, 292/296, 292/296/300, 296, and

296/300, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 94.

5. The recombinant lipase of Claim 1, wherein said recombinant lipase comprises a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 350, and wherein said polypeptide sequence comprises at least one substitution or substitution set at one or more positions selected from 4, 4/27/189/300, 4/70, 4/102, 4/137, 4/175/189/218, 4/175/189/300, 4/175/218/224, 4/175/218/373, 4/185, 4/189/218/373, 4/193, 4/218/300/369/373, 4/228, 4/233, 4/243, 4/271, 4/303, 4/334, 4/336, 4/375, 25, 27/82/174/175/189/218/300/369/373, 27/82/174/175/218/300/369, 27/82/174/175/218/300/373/382, 27/82/174/218/300/373, 27/189/218/373, 28, 33, 99, 102, 104, 134, 153, 174/175/189, 174/373, 175/189/218/300/373, 175/189/373, 175/218, 175/218/382, 189/218/300/373, 191, 193, 231, 233, 243, 293, 303, 331, 336, 339, 368, and 373, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 350.

6. The recombinant lipase of Claim 1, wherein said recombinant lipase comprises a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 442, and wherein said polypeptide sequence comprises at least one substitution or substitution set at one or more positions selected from 26, 33, 33/174/193/243, 33/174/334, 33/175/218/303, 33/175/218/334/339, 33/193, 34, 38, 46, 48, 70/271/293/334, 144, 174, 174/175/193, 174/175/218/339, 174/193/303/375, 174/193/375, 174/218/233/271/293/303, 174/218/271/303, 175/193/218/233/243/375, 175/193/218/243, 175/218/375, 181, 189, 193, 193/218/243, 193/271/303/334, 193/293/303, 194, 195, 199, 210, 218, 218/243, 218/243/303/334, 218/303, 225, 238, 274, 281, and 330, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 442.

7. The recombinant lipase of Claim 1, wherein said recombinant lipase comprises a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 540, and wherein said polypeptide sequence comprises at least one substitution or substitution set at one or more positions selected from 34/38/174/193/195/243/281/303/330, 34/38/174/225/303, 34/38/174/303/345, 34/174, 34/174/193/195/243/281, 34/174/193/195/303/330, 34/193/195/225/243, 34/193/243/303/330, 34/281/330, 38, 38/174/193/195/243/330, 38/174/193/225/274/283/303/330, 38/174/193/303, 38/174/195/281/330, 38/174/225/243/281/330, 38/174/281, 38/174/281/303, 38/174/281/303/345, 38/193/195/225/303/345, 38/195/243/303, 38/195/281/303/330, 38/195/281/330, 38/195/303, 49, 49/51/98/252/311, 49/51/123/252/344, 49/98/120/252/344, 49/120/252/344, 49/123/252/344, 49/123/264/344, 49/123/311, 49/252/344, 49/311/344, 49/344, 51, 51/252/344, 51/344, 98, 98/344,

123/252/344, 129, 160, 161, 174/193/195/225, 174/193/303/330, 174/195/225/281/303/330/345, 174/195/243/281/345, 174/195/281/303, 174/225/243/281/303/345, 174/281/330, 174/303, 195/225/303/330, 252, 268, and 344, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 540.

8. The recombinant lipase of Claim 1, wherein said recombinant lipase comprises a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 646, and wherein said polypeptide sequence comprises at least one substitution or substitution set at one or more positions selected from 11/16/67/168/180/287, 11/16/237/241/287, 11/67/168/287, 16/67/154/168/237/241/287, 16/67/237/287/291, 16/168/177, 24, 24/278, 34/49/161/193/243/344, 34/49/161/193/344, 34/49/161/243/344, 34/49/161/252/268/344, 34/49/193/243/252/344, 34/49/193/344, 34/49/243/252, 34/49/252/268/344, 34/161/193/243/252/268, 34/161/193/243/268/344, 34/161/193/243/344, 34/161/193/252/268/344, 34/161/193/252/344, 34/161/193/268/344, 34/161/243/281/344, 34/161/344, 34/193/243/252/344, 34/193/252/268/344, 34/193/268/281, 34/252/268/281/344, 34/252/344, 49/161/193/243/252/344, 49/161/193/252/281/344, 49/161/243/252/344, 49/193/197/243/252/281/344, 49/193/243/252/344, 49/193/243/344, 49/243/344, 67/154/237/287, 67/168/237, 67/168/237/287, 67/177/237, 154/237/286/287, 154/286/287, 161/193/252/344, 161/193/344, 161/344, 168/237, 186/187/278, 193/243/281/344, 193/252/268/344, 193/252/281/344, 237, 237/287/291, and 281/344, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 646.

9. The recombinant lipase of Claim 1, wherein said recombinant lipase comprises a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 758, and wherein said polypeptide sequence comprises at least one substitution or substitution set at one or more positions selected from 24/168/252/281, 24/237/287/344, 24/252, 24/252/287, 24/344, 213/252/278/344, 252/278/344, 252/287/344, and 281, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 758.

10. The recombinant lipase of Claim 1, wherein said recombinant lipase comprises a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 868, and wherein said polypeptide sequence comprises at least one substitution or substitution set at one or more positions selected from 24, 24/38/49/70/149/161/174/175/189/193/225/231/243/252/271/287/292/293/296/303/334/344/373/385, 38, 49, 70, 149, 161, 174, 175, 189, 193, 225, 231, 243, 252, 271, 287, 292, 293, 296, 303, 334, 344,

373, and 385, wherein the amino acid positions of the polypeptide sequence are numbered with reference to SEQ ID NO: 868.

11. The recombinant lipase of Claim 1, wherein said recombinant lipase comprises at least one mutation in at least one position as provided in Tables 4-1, 4-2, 4-3, 4-4, 4-5, 4-6, 4-7, and/or 5-1, wherein the positions are numbered with reference to SEQ ID NO: 2, 94, 350, 442, 540, 646, 758, and/or 868.

12. The recombinant lipase of any of Claims 1-11, wherein said recombinant lipase is more thermostable than the lipase of SEQ ID NO: 2, 94, 350, 442, 540, 646, 758, and/or 868.

13. The recombinant lipase of any of Claims 1-12, wherein said recombinant lipase is more stable at acidic pH levels, than the lipase of SEQ ID NO: 2, 94, 350, 442, 540, 646, 758, and/or 868.

14. The recombinant lipase of any of Claims 1-13, wherein said recombinant lipase is more resistant to proteolysis than the lipase of SEQ ID NO: 2, 94, 350, 442, 540, 646, 758, and/or 868.

15. The recombinant lipase of any of Claims 1-14, wherein said recombinant lipase is more active in the presence of at least one bile salt than the lipase of SEQ ID NO: 2, 94, 350, 442, 540, 646, 758, and/or 868.

16. The recombinant lipase of any of Claims 1-15, wherein said recombinant lipase is more active in the presence of at least one bile salt than the lipase of SEQ ID NO: 2, 94, 350, 442, 540, 646, 758, and/or 868.

17. The recombinant lipase of any of Claims 1-16, wherein said recombinant lipase is purified.

18. The recombinant lipase of any of Claims 1-17, wherein said recombinant lipase exhibits at least one improved property selected from: i) enhanced catalytic activity; ii) increased tolerance to acid pH; iii) increased tolerance to pH 3.5; iv) increased tolerance to pH 3; v) increased tolerance to at least one protease; vi) increased tolerance to at least one bile salt; vii) increased thermotolerance; or a combination of any of i), ii), iii), iv), v), vi), and vii) as compared to a reference sequence.

19. The recombinant lipase of Claim 18, wherein said reference sequence is selected from SEQ ID NO: 2, 94, 350, 442, 540, 646, 758, and 868.
20. A composition comprising at least one recombinant lipase of any of Claims 1-19.
21. A recombinant polynucleotide sequence encoding at least one recombinant lipase set forth in any of Claims 1-19.
22. The recombinant polynucleotide sequence of Claim 21, wherein said polynucleotide sequence is codon-optimized.
23. An expression vector comprising at least one recombinant polynucleotide sequence of Claim 21 and/or 22.
24. The expression vector of Claim 23, wherein said recombinant polynucleotide sequence is operably linked to a control sequence.
25. The expression vector of Claim 23 or 24, wherein said control sequence is a promoter.
26. The expression vector of Claim 25, wherein said promoter is a heterologous promoter.
27. A host cell comprising the expression vector of any of Claims 23-26.
28. The host cell of Claim 27, wherein said host cell is eukaryotic or prokaryotic.
29. A method of producing a recombinant lipase, comprising culturing said host cell of Claim 27 or 28, under conditions that said recombinant lipase encoded by said recombinant polynucleotide is produced.
30. The method of Claim 29, further comprising the step of recovering said lipase.
31. The method of Claim 30, further comprising the step of purifying said lipase.
32. A pharmaceutical composition for the treatment of pancreatic insufficiency, comprising the composition of Claim 20.

33. The pharmaceutical composition of Claim 32, further comprising a pharmaceutically acceptable carrier and/or excipient.

34. The composition of Claim 32 and/or 33, further comprising at least one additional enzyme selected from at least one protease and at least one amylase.

35. The composition of Claim 32 and/or 33, further comprising a pancreatic extract, containing lipase, protease, and amylase activity.

36. The pharmaceutical composition of any of Claims 32 to 35, wherein said composition is suitable for parenteral injection or infusion, subcutaneous injection, inhalation, or dermal application to a human.

37. The pharmaceutical composition of any of Claims 32 to 35, wherein said composition is suitable for oral administration to a human.

38. The pharmaceutical composition of Claim 37, wherein said composition further comprises an enteric coating.

39. A method for treating and/or preventing the symptoms of pancreatic insufficiency in a subject, comprising providing a subject having pancreatic insufficiency, and providing the pharmaceutical composition of any of Claims 32-38, to said subject.

40. The method of Claim 39, wherein said symptoms of pancreatic insufficiency are ameliorated.

41. The method of Claim 39 and/or 40, wherein said subject is able to eat a diet that is less restricted in its lipid content than diets required by subjects exhibiting the symptoms of pancreatic insufficiency.

42. The method of any of Claims 39-41, wherein said subject is an infant or child.

43. The method of any of Claims 39-41, wherein said subject is an adult or young adult.

44. A recombinant lipase according to any of Claims 1-19, or a composition according to any of Claims 20 and 32-38, for use as a medicament.

45. A recombinant lipase according to any of Claims 1-19, or a composition according to any of Claims 20 and 32-38, for use as a nutritional supplement.

46. A recombinant lipase according to any of Claim 1-19, or a composition according to any of Claims 20 and 32-38, for use in the treatment, or prevention of, symptoms of pancreatic insufficiency.

47. Use of the compositions provided in any of Claims 20, and 32-38.