CLAIMS

What is claimed is:

1. An engineered ketoreductase variant having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 4, and at least one substitution or substitution set at one or more positions selected from positions 12, 21, 87, 93, 97, 110, 145, 148, 152, 153, 194, 196, 197, 200, 206, 212, and 226, wherein said positions are numbered with reference to SEQ ID NO: 4.

2. An engineered ketoreductase variant having:

a) at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 6, and at least one substitution or substitution set selected from 12/110/145/152, 12/145, 87/110/145, 87/110/145/194, 87/145/194, 110, 110/145/152/197, 110/145/194, 145, 145/152, 145/197/226, and 152, wherein said positions are numbered with reference to SEQ ID NO: 6; or

b) least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 80, and at least one substitution or substitution set selected from 17, 21, 46, 56, 72, 79, 95, 101, 110, 152, 162, 190, 198, 210, 211, and 227, wherein said positions are numbered with reference to SEQ ID NO: 80; or

c) at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 80, and at least one substitution or substitution set selected from 17, 79, 157, 159, 190/191/194, 190/194, 191/194, 194, 198, and 211, wherein said positions are numbered with reference to SEQ ID NO: 80; or

d) at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 104, and at least one substitution or substitution set selected from 17/46/190, 17/46/198/211, 17/96/194/198, 17/190/198, 46/190/194/198, and 46/194/198, wherein said positions are numbered with reference to SEQ ID NO: 104; or

e) at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 172, and at least one substitution or substitution set selected from 45, 101,

179, 194, 204, 226, and 231, wherein said positions are numbered with reference to SEQ ID NO: 172; or

f) at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 186, and at least one substitution or substitution set selected from 95/96/97/150/153/205, 95/96/150/153/205/206/211/249, 95/97/143/145/150/153/202/205, 95/97/143/145/150/153/249, 95/97/150/153, 95/97/150/153/202/205/206, 95/150/153/205/206/211, 95/150/153/205/211, 95/150/153/206/249, 96/150/153, 96/150/153/206, 97/150/153, 97/150/153/205, 97/150/153/205/211, 97/150/153/206, 143/144/145/150/153/202/205/249, 143/145/150/153, 144/145/150/153/205/206, 144/150/153, 144/150/153/202/205/206, 145/150/153/206/249, 145/153/211, 150/153/202/206/249, 150/153/205/211, 150/153/206/211, 150/153/211, and 150/153/249, wherein said positions are numbered with reference to SEQ ID NO: 186; or

g) at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 186, and at least one substitution or substitution set selected from 7/147, 103/147, 110, 110/179/194, 147, and 249, wherein said positions are numbered with reference to SEQ ID NO: 186; or

h) at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 194, and at least one substitution or substitution set selected from 7/12/54/110/150/153/194/205/211/249, 12/54/72/110/150/152/153/194/205/211/249, 12/72/101/103/110/152/249, 12/72/110/147/152/204, 45/54/72/110/152/194/204, 72/110/147/150/152/153/194/205/211/249, and 110/150/153/179/194/205/211/249, wherein said positions are numbered with reference to SEQ ID NO: 194; or

i) at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 252, and at least one substitution or substitution set selected from 7/12/54/179/249, 7/152, 12/54/72/152/179/249, 40, 54/72, 72/147/152/179/249, and 249, wherein said positions are numbered with reference to SEQ ID NO: 252; or

j) at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 270, and at least one substitution or substitution set selected from 92/93, 150/152, 150/152/153, and 194/195, wherein said positions are numbered with reference to SEQ ID NO: 270; or

k) at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 272, and at least one substitution or substitution set selected from 92/93/95,

93, 93/95, 93/95/109, 93/95/109/114, 93/95/114, 93/109/114, and 114, wherein said positions are numbered with reference to SEQ ID NO: 272; or

l) at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 286, and at least one substitution or substitution set selected from 12/45/72/109/249, 12/45/93/249, 12/45/249, 12/109/249, 45/72/249, 45/109/249, 45/249, 96, and 145/150, wherein said positions are numbered with reference to SEQ ID NO: 286.

3. An engineered ketoreductase variant having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 328, and at least one substitution or substitution set selected from 150, 150/151, 150/195, and 195, wherein said positions are numbered with reference to SEQ ID NO: 328.

4. An engineered ketoreductase variant having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 330, and at least one substitution or substitution set selected from 12/72/109/195, 17/73/200, 17/115, 68/72/101/152/205, 68/72/124, 68/72/124/152, 68/101/124/152/205, 68/124/205, 72/109/152/195, 72/109/195, 72/152, 72/152/195, 72/195, 73, 73/147, 79, 93, 93/95/145/195, 93/109/114/145/195, 93/195, 95/195, 96/108/147/200, 96/194/200, 101/205, 145/195, 147, 147/200, 192, 194, 194/200, 195, 198, and 200, wherein said positions are numbered with reference to SEQ ID NO: 330.

5. The engineered ketoreductase variant of any one of Claims 1 to 4, comprising a polypeptide sequence having at least 90% sequence identity to SEQ ID NO: 4, 6, 80, 104, 172, 186, 194, 252, 270, 272, 286, 328, and/or 330.

6. The engineered ketoreductase variant of any one of Claims 1 to 5, comprising a polypeptide sequence having at least 95% sequence identity to SEQ ID NO: 4, 6, 80, 104, 172, 186, 194, 252, 270, 272, 286, 328, and/or 330.

7. The engineered ketoreductase variant of any one of Claims 1 to 6, comprising a polypeptide sequence set forth in SEQ ID NO: 6, 80, 104, 172, 186, 194, 252, 270, 272, 286, 328, and/or 330.

8. The engineered ketoreductase variant of any one of Claims 1 to 7, wherein said engineered ketoreductase comprises a polypeptide sequence encoding a variant provided in Table 5-1, 6-1, 7-1, 8-1, 10-1, 11-1, 16-1, 17-2, 18-1, 19-1, 19-2, 20-1, 20-2, 21-1, 22-1 or 24-1.

9. The engineered ketoreductase variant of any one of Claims 1 to 8, wherein said engineered ketoreductase comprises a polypeptide sequence selected from the even-numbered sequences set forth in SEQ ID NOS: 6 to 412.

10. The engineered ketoreductase variant of Claim 1, wherein the engineered ketoreductase polypeptide is capable of converting one or more iso-α-acid substrates to one or more corresponding dihydro-(rho)-iso-α-acid products.

11. The engineered ketoreductase variant of Claim 1 which is capable of converting one or more iso-α-acid substrates to one or more corresponding dihydro-(rho)-iso-α-acid products with at least 10-fold the activity of the reference polypeptide of SEQ ID NO:4.

12. The engineered ketoreductase variant of any one of Claims 1 to 11, wherein said engineered ketoreductase comprises an improved property as compared to the ketoreductase of SEQ ID NO: 4.

13. The engineered ketoreductase variant of Claim 12, wherein the improved property comprises improved activity converting iso-α-acids to the corresponding dihydro-(rho)-iso-α-acids as compared to the ketoreductase of SEQ ID NO: 4.

14. The engineered ketoreductase variant of Claim 12, wherein the improved property comprises improved activity at high substrate concentrations as compared to the ketoreductase of SEQ ID NO: 4.

15. The engineered ketoreductase variant of Claim 12, wherein the improved property comprises improved activity at low cofactor concentrations as compared to the ketoreductase of SEQ ID NO: 4.

16. An engineered polynucleotide sequence encoding the engineered ketoreductase variant of any one of Claims 1 to 15.

17. The engineered polynucleotide sequence of Claim 16, wherein said sequence comprises a polynucleotide sequence that is at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a sequence selected from the odd-numbered sequences set forth in SEQ ID NOS: 5 to 411.

18. A vector comprising the engineered polynucleotide sequence of Claim 16 or 17, optionally comprising at least one control sequence

19. The vector of Claim 18 , wherein said vector comprises SEQ ID NO: 327, 413 or 414.

20. A host cell comprising the vector of Claim 19.

21. A method for producing the engineered ketoreductase variant of any one of Claims 1 to 15, comprising culturing said host cell of Claim 20 under conditions that said engineered ketoreductase variant is produced by said host cell, optionally comprising the step of recovering said engineered ketoreductase variant produced by said host cell.

22. The method of Claim 21, wherein the engineered ketoreductase variant is produced by a host cell comprising the vector of SEQ ID NO: 327, 413 and/or 414.

23. A composition comprising at least one engineered ketoreductase variant provided in any one of Claims 1 to 15.