

CLAIMS

What is claimed is:

1. An engineered polypeptide having proline hydroxylase activity comprises an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO:4 and one or more residue differences as compared to SEQ ID NO:4 at residue positions selected from: 2, 4, 8, 10, 15, 26, 30, 33, 36, 37, 39, 42, 43, 44, 45, 48, 50, 52, 55, 56, 57, 58, 61, 62, 63, 71, 76, 77, 81, 82, 87, 88, 92, 94, 95, 97, 98, 101, 107, 109, 114, 115, 119, 121, 124, 128, 130, 131, 132, 134, 136, 145, 151, 153, 156, 158, 160, 161, 165, 166, 168, 173, 176, 178, 180, 184, 194, 213, 230, 237, 240, 256, 263, 266, 269, 270, 271, 273, 274, 275, and 280.
2. The engineered polypeptide of Claim 1, wherein said polypeptide has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to at least one of the even-numbered sequences in SEQ ID NOS:6-1004.
3. An engineered polypeptide having proline hydroxylase activity comprises an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO:604 and one or more residue differences as compared to SEQ ID NO:604 at residue positions selected from: 13, 14, 24, 26, 27, 30, 57, 61, 62, 72, 76, 77, 81, 82, 86, 88, 97, 114, 127, 128, 142, 158, 161, 163, 173, 175, 176, 178, 180, 184, 185, 186, 187, 188, 189, 191, 192, 195, 198, 200, 207, 209, 210, 211, 213, 215, 217, 218, 222, 225, 230, 233, 236, 238, 240, 241, 256, 259, 263, 265, 271, and 273.
4. The engineered polypeptide of Claim 3, wherein said polypeptide has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to at least one of the even-numbered sequences in SEQ ID NOS:6-1004.
5. An engineered polypeptide having proline hydroxylase activity comprises an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO:810 and one or more residue differences as compared to SEQ ID NO:810 at residue positions selected from: 33, 40, 95, and 156.
6. The engineered polypeptide of Claim 5, wherein said polypeptide has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to at least one of the even-numbered sequences in SEQ ID NOS:6-1004.

7. The engineered polypeptide of any one of Claims 1 to 6, wherein said engineered polypeptide is capable of converting (S)-pipecolic acid to (2S,5S)-5-hydroxypipecolic acid.
8. The engineered polypeptide of Claim 7, wherein said engineered polypeptide is capable of converting (S)-pipecolic acid to (2S,5S)-5-hydroxypipecolic acid with at least 1.2 fold, 1.5 fold, 2 fold, 3 fold, 4 fold, 5 fold, 10 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, 100 fold or more the activity of the naturally occurring enzyme.
9. The engineered polypeptide of Claim 7 or 8, wherein said engineered polypeptide is capable of converting (S)-pipecolic acid to (2S,5S)-5-hydroxypipecolic acid with greater than 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99 % or more diastereomeric excess of (2S,5R)-5-hydroxypipecolic acid.
10. A polynucleotide encoding the engineered polypeptide of any one of Claims 1 to 9.
11. The polynucleotide of Claim 10, wherein said polynucleotide comprises a nucleic acid sequence optimized for expression in *E. coli*.
12. An expression vector comprising the polynucleotide of Claim 10 or 11, optionally further comprising at least one control sequence.
13. The expression vector of Claim 12, wherein said vector comprises SEQ ID NO:1007, 1008, or 1009.
14. A host cell comprising the polynucleotide of any of Claims 10-13.
15. The host cell of Claim 14, wherein the host cell is *E. coli*.
16. A method of preparing the engineered polypeptide of any one of Claims 1 to 9, comprising culturing the host cell of Claim 14 or 15 under conditions suitable for expression of the polypeptide.
17. The method of Claim 16, further comprising a step of isolating the engineered polypeptide.