CLAIMS

1. An engineered carboxyesterase comprising a polypeptide sequence having at least 80% sequence identity to SEQ ID NO: 2, wherein said engineered carboxyesterase comprises a substitution in said polypeptide sequence at position 282, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2, and wherein said engineered carboxyesterase exhibits greater enzymatic activity than wild-type *T. fusca* carboxyesterase of SEQ ID NO: 2.

2. The engineered carboxyesterase of Claim 1, wherein said polypeptide sequence further comprises substitutions at positions selected from: 39, 39/323, 62, 62/117, 63, 64, 65, 66, 68, 69, 70, 71, 71/263, 77, 77/184, 103, 103/147, 104, 104/429, 105, 107, 107/185, 108, 109, 109/117, 110, 111, 113, 114, 115, 117, 118, 118/269, 118/349, 119, 126, 147, 153, 153/215, 164, 164/271, 174, 183, 184, 184/249, 185, 186, 187, 188, 190, 209, 210, 211, 212, 213, 213/271, 213/345, 214, 215, 215/271, 216, 217, 217/231, 224, 224/268/372, 231, 249, 249/284, 263, 268, 269, 270, 270/470, 271, 271/416, 276, 277, 278, 279, 280, 281, 281/374, 283, 283/429, 284, 284/438, 285, 286, 311, 317, 320, 320/323, 320/323/372, 320/372/376, 320/376/377, 321, 323, 324, 345, 349, 372, 372/376, 373, 374, 376, 377, 405, 416, 420, 427, 428, 429, 438, and 470, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2.

3. The engineered carboxyesterase of Claim 1, wherein said amino acid at position 282 is a polar amino acid.

4. The engineered carboxyesterase of Claim 1, wherein said engineered carboxyesterase comprises an amino acid substitution selected from: X282Q, X282S, and X282T, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2.

5. The engineered carboxyesterase of Claim 1, wherein said polypeptide sequence is selected from: SEQ ID NOs: 4, 6, 8, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132 and 134.

6. The engineered carboxyesterase of Claim 1, wherein said engineered carboxyesterase comprises improved amidation activity as compared to the wild-type *T. fusca* carboxyesterase of SEQ ID NO:2.

7. The engineered carboxyesterase of Claim 1, wherein said engineered carboxyesterase exhibits greater enzymatic activity than wild-type *T. fusca* carboxyesterase on a substrate comprising isobutylamine.

8. The engineered carboxyesterase of Claim 1, wherein said engineered carboxyesterase exhibits greater enzymatic activity than wild-type *T. fusca* carboxyesterase on the substrate set comprising isobutylamine and ethyl benzoate.

9. The engineered carboxyesterase of Claim 1, wherein said engineered carboxyesterase exhibits greater enzymatic activity than wild-type *T. fusca* carboxyesterase in producing a product comprising *N*-isobutyl-benzamide.

10. The engineered carboxyesterase of Claim 1, further comprising at least one substitution selected from: L343V, L372L, L320W/G, L214R, L271Y and L65G, wherein said engineered carboxyesterase exhibits greater enzymatic activity than wild-type *T. fusca* carboxyesterase, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2.

11. The engineered carboxyesterase of Claim 1, further comprising at least one substitution selected from: L268A, L63A/R, L189Q/I/E, L214R, L381L and L69W, wherein said engineered carboxyesterase exhibits greater enzymatic activity than wild-type *T. fusca* carboxyesterase, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2.

12. The engineered carboxyesterase of Claim 1, wherein said engineered carboxyesterase is purified.

13. The engineered carboxyesterase of Claim 1, wherein said engineered carboxyesterase is immobilized on a solid support.

14. A composition comprising at least one engineered carboxyesterase of Claim 1.

ABSTRACT

The present invention provides engineered carboxyesterase enzymes having improved properties as compared to a naturally occurring wild-type carboxyesterase enzymes, as well as polynucleotides encoding the engineered carboxyesterase enzymes, host cells capable of expressing the engineered carboxyesterase enzymes, and methods of using the engineered carboxyesterase enzymes in amidation reactions.

[NO FIGURE]