

CLAIMS

1. An engineered carboxyesterase comprising a polypeptide sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 2 or a functional fragment thereof, wherein said engineered carboxyesterase comprises at least one substitution or substitution set in said polypeptide sequence, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2.

2. The engineered carboxyesterase of Claim 1, wherein said engineered carboxyesterase comprises one or more amino acid substitutions at positions selected from: X9, X10, X70, X82, and X180.

3. The engineered carboxyesterase of Claim 1, wherein said at least one substitution or substitution set in said polypeptide sequence comprises substitutions at positions selected from: 3/4/6/9/10/11/36/38/70/82/180/200/214/229, 3/6/9/10/70/99, 3/8/9/10/11/13/70/82/107/229, 3/8/9/10/11/13/70/107, 3/8/9/10/11/70, 3/8/9/10/11/70/229, 3/8/9/10/13/70/82/107, 3/8/9/10/13/70/82/229, 3/8/9/10/70/82, 3/8/9/10/70/82/229, 3/8/9/10/70/229, 3/9/10/11/13/82, 3/9/10/11/70/82/107, 3/9/10/11/70/229, 3/9/10/70/82, 3/9/11, 3/9/11/70/82/180, 4/6/8/9/10/11/38/70/82/180/186/200/214/229, 4/6/9/10/11/36/70/82/180/200/214/229, 4/6/9/10/11/38/70/82/180/186/200/214, 4/6/9/10/70/82/229, 4/8/9/10/11/13/70/82/107/229, 4/8/9/10/11/36/38/70/82/180/200/214/229, 4/8/9/10/11/36/70/82/180/186/200/214/229, 4/8/9/10/11/70/82/180/200/214/229, 4/8/9/10/36/38/70/82/180/200/214/229, 4/9/10/11/36/38/70/82/180/200/214/229, 4/9/10/11/36/65/70/82/180/186/200/214, 4/9/10/11/36/70/82/180/200/214, 4/9/10/11/36/70/82/180/200/214/229, 4/9/10/11/70/82/180/200/214, 6/8/9/10/38/70/82/180/200/214, 6/9/10/11/36/70/82/180/200/214/229, 6/9/10/38/70/82/180/186/200/214/229, 6/9/10/70/82/180/186/200/214/229, 8/9/10, 8/9/10/11/13/70, 8/9/10/11/13/70/82, 8/9/10/11/13/70/82/107/229, 8/9/10/11/36/38/70/82/180/200/214, 8/9/10/11/38/70/82/180/200/214/229, 8/9/10/11/43/70/82/180/200/214, 8/9/10/11/70, 8/9/10/11/70/82/107/229, 8/9/10/11/70/229, 8/9/10/70, 8/9/10/70/82/229, 9/10, 9/10/11/12/49/51/70/73/82/180/200/225, 9/10/11/12/49/70/73/82/180, 9/10/11/12/51/54/70/73/82/108/148/180/225, 9/10/11/12/51/54/70/73/82/110/180, 9/10/11/12/51/54/70/73/82/148/180/225, 9/10/11/12/51/70/73/82/148/180, 9/10/11/12/51/70/73/82/148/180/200/225, 9/10/11/12/51/70/73/110/148/180, 9/10/11/17/49/70/82/180/200, 9/10/11/17/49/70/82/180/219, 9/10/11/17/51/54/70/82/148/180/200, 9/10/11/17/51/54/70/82/180, 9/10/11/17/70/73/82/87/180/219, 9/10/11/17/70/82/148/180/219, 9/10/11/35/70/82/180/200/214, 9/10/11/36/38/65/70/82/180/200/214/229,

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4. An engineered carboxyesterase comprising a polypeptide sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 82 or a functional fragment thereof, wherein said engineered carboxyesterase comprises at least one substitution or substitution set in said polypeptide sequence, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 82.

5. The engineered carboxyesterase of Claim 4, wherein said at least one substitution or substitution set in said polypeptide sequence comprises substitutions at positions selected from: 3/4/6/9/10/11/36/38/70/82/180/200/214/229, 3/6/9/10/70/99, 3/8/9/10/11/13/70/82/107/229, 3/8/9/10/11/13/70/107, 3/8/9/10/11/70, 3/8/9/10/11/70/229, 3/8/9/10/13/70/82/107, 3/8/9/10/13/70/82/229, 3/8/9/10/70/82, 3/8/9/10/70/82/229, 3/8/9/10/70/229, 3/9/10/11/13/82, 3/9/10/11/70/82/107, 3/9/10/11/70/229, 3/9/10/70/82, 3/9/11, 3/9/11/70/82/180, 4/6/8/9/10/11/38/70/82/180/186/200/214/229, 4/6/9/10/11/36/70/82/180/200/214/229, 4/6/9/10/11/38/70/82/180/186/200/214, 4/6/9/10/70/82/229, 4/8/9/10/11/13/70/82/107/229, 4/8/9/10/11/36/38/70/82/180/200/214/229, 4/8/9/10/11/36/70/82/180/186/200/214/229, 4/8/9/10/11/70/82/180/200/214/229, 4/8/9/10/36/38/70/82/180/200/214/229, 4/9/10/11/36/38/70/82/180/200/214/229, 4/9/10/11/36/65/70/82/180/186/200/214, 4/9/10/11/36/70/82/180/200/214, 4/9/10/11/36/70/82/180/200/214/229, 4/9/10/11/70/82/180/200/214, 6/8/9/10/38/70/82/180/200/214, 6/9/10/11/36/70/82/180/200/214/229,

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6. An engineered carboxyesterase comprising a polypeptide sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% more sequence identity to SEQ ID NO: 268 or a functional fragment thereof, wherein said engineered carboxyesterase comprises at least one substitution or substitution set in said polypeptide sequence, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 268.

7. The engineered carboxyesterase of Claim 6, wherein said at least one substitution or substitution set in said polypeptide sequence comprises substitutions at positions selected from:

3/4/6/9/10/11/36/38/70/82/180/200/214/229, 3/6/9/10/70/99, 3/8/9/10/11/13/70/82/107/229, 3/8/9/10/11/13/70/107, 3/8/9/10/11/70, 3/8/9/10/11/70/229, 3/8/9/10/13/70/82/107, 3/8/9/10/13/70/82/229, 3/8/9/10/70/82, 3/8/9/10/70/82/229, 3/8/9/10/70/229, 3/9/10/11/13/82, 3/9/10/11/70/82/107, 3/9/10/11/70/229, 3/9/10/70/82, 3/9/11, 3/9/11/70/82/180, 4/6/8/9/10/11/38/70/82/180/186/200/214/229, 4/6/9/10/11/36/70/82/180/200/214/229, 4/6/9/10/11/38/70/82/180/186/200/214, 4/6/9/10/70/82/229, 4/8/9/10/11/13/70/82/107/229, 4/8/9/10/11/36/38/70/82/180/200/214/229, 4/8/9/10/11/36/70/82/180/186/200/214/229, 4/8/9/10/11/70/82/180/200/214/229, 4/8/9/10/36/38/70/82/180/200/214/229, 4/9/10/11/36/38/70/82/180/200/214/229, 4/9/10/11/36/65/70/82/180/186/200/214, 4/9/10/11/36/70/82/180/200/214, 4/9/10/11/36/70/82/180/200/214/229, 4/9/10/11/70/82/180/200/214, 6/8/9/10/38/70/82/180/200/214, 6/9/10/11/36/70/82/180/200/214/229, 6/9/10/38/70/82/180/186/200/214/229, 6/9/10/70/82/180/186/200/214/229, 8/9/10, 8/9/10/11/13/70, 8/9/10/11/13/70/82, 8/9/10/11/13/70/82/107/229, 8/9/10/11/36/38/70/82/180/200/214, 8/9/10/11/38/70/82/180/200/214/229, 8/9/10/11/43/70/82/180/200/214, 8/9/10/11/70, 8/9/10/11/70/82/107/229, 8/9/10/11/70/229, 8/9/10/70, 8/9/10/70/82/229, 9/10, 9/10/11/12/49/51/70/73/82/180/200/225, 9/10/11/12/49/70/73/82/180, 9/10/11/12/51/54/70/73/82/108/148/180/225, 9/10/11/12/51/54/70/73/82/110/180, 9/10/11/12/51/54/70/73/82/148/180/225, 9/10/11/12/51/70/73/82/148/180, 9/10/11/12/51/70/73/82/148/180/200/225, 9/10/11/12/51/70/73/110/148/180, 9/10/11/17/49/70/82/180/200, 9/10/11/17/49/70/82/180/219, 9/10/11/17/51/54/70/82/148/180/200, 9/10/11/17/51/54/70/82/180, 9/10/11/17/70/73/82/87/180/219, 9/10/11/17/70/82/148/180/219, 9/10/11/35/70/82/180/200/214, 9/10/11/36/38/65/70/82/180/200/214/229, 9/10/11/36/38/70/82/180/200/214, 9/10/11/36/38/70/82/180/200/214/229, 9/10/11/36/70/82/180/196/214, 9/10/11/36/70/82/180/200/214, 9/10/11/36/70/82/180/200/214/229, 9/10/11/38/70/82/180/186/200/214, 9/10/11/40/70/82/180/200/214, 9/10/11/49/51/54/70/82/87/108/110/148/180/219, 9/10/11/49/51/54/70/82/87/108/180, 9/10/11/49/51/54/70/82/110/148/180, 9/10/11/49/51/70/82/108/110/148/180, 9/10/11/49/54/70/82/87/148/180, 9/10/11/49/70/82/87/108/110/180/200, 9/10/11/49/70/82/87/180, 9/10/11/49/70/82/110/148/180/225, 9/10/11/51/54/70/73/82/108/110/180, 9/10/11/51/54/70/73/82/108/180/200/225, 9/10/11/51/54/70/73/82/110/148/180, 9/10/11/51/54/70/82/148/180, 9/10/11/51/70/82/110/180/192/219, 9/10/11/54/70/82/87/108/180, 9/10/11/54/70/82/87/180/192, 9/10/11/54/70/82/148/180/200, 9/10/11/54/70/82/180/200/214, 9/10/11/70, 9/10/11/70/71/82/180/200/214, 9/10/11/70/73/82/87/110/180, 9/10/11/70/73/82/108/148/180, 9/10/11/70/73/82/148/180, 9/10/11/70/73/82/148/180/192/200,

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8. An engineered carboxyesterase comprising a polypeptide sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 536 or a functional fragment thereof, wherein said engineered carboxyesterase comprises at least one substitution or substitution set in said polypeptide sequence, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 536.

9. The engineered carboxyesterase of Claim 8, wherein said at least one substitution or substitution set in said polypeptide sequence comprises substitutions at positions selected from: 3/4/6/9/10/11/36/38/70/82/180/200/214/229, 3/6/9/10/70/99, 3/8/9/10/11/13/70/82/107/229, 3/8/9/10/11/13/70/107, 3/8/9/10/11/70, 3/8/9/10/11/70/229, 3/8/9/10/13/70/82/107, 3/8/9/10/13/70/82/229, 3/8/9/10/70/82, 3/8/9/10/70/82/229, 3/8/9/10/70/229, 3/9/10/11/13/82, 3/9/10/11/70/82/107, 3/9/10/11/70/229, 3/9/10/70/82, 3/9/11, 3/9/11/70/82/180, 4/6/8/9/10/11/38/70/82/180/186/200/214/229, 4/6/9/10/11/36/70/82/180/200/214/229, 4/6/9/10/11/38/70/82/180/186/200/214, 4/6/9/10/70/82/229, 4/8/9/10/11/13/70/82/107/229, 4/8/9/10/11/36/38/70/82/180/200/214/229, 4/8/9/10/11/36/70/82/180/186/200/214/229, 4/8/9/10/11/70/82/180/200/214/229, 4/8/9/10/36/38/70/82/180/200/214/229, 4/9/10/11/36/38/70/82/180/200/214/229, 4/9/10/11/36/65/70/82/180/186/200/214, 4/9/10/11/36/70/82/180/200/214, 4/9/10/11/36/70/82/180/200/214/229, 4/9/10/11/70/82/180/200/214, 6/8/9/10/38/70/82/180/200/214, 6/9/10/11/36/70/82/180/200/214/229, 6/9/10/38/70/82/180/186/200/214/229, 6/9/10/70/82/180/186/200/214/229, 8/9/10, 8/9/10/11/13/70, 8/9/10/11/13/70/82, 8/9/10/11/13/70/82/107/229, 8/9/10/11/36/38/70/82/180/200/214, 8/9/10/11/38/70/82/180/200/214/229, 8/9/10/11/43/70/82/180/200/214, 8/9/10/11/70, 8/9/10/11/70/82/107/229, 8/9/10/11/70/229, 8/9/10/70, 8/9/10/70/82/229, 9/10, 9/10/11/12/49/51/70/73/82/180/200/225, 9/10/11/12/49/70/73/82/180, 9/10/11/12/51/54/70/73/82/108/148/180/225, 9/10/11/12/51/54/70/73/82/110/180, 9/10/11/12/51/54/70/73/82/148/180/225, 9/10/11/12/51/70/73/82/148/180, 9/10/11/12/51/70/73/82/148/180/200/225, 9/10/11/12/51/70/73/110/148/180, 9/10/11/17/49/70/82/180/200, 9/10/11/17/49/70/82/180/219, 9/10/11/17/51/54/70/82/148/180/200, 9/10/11/17/51/54/70/82/180, 9/10/11/17/70/73/82/87/180/219, 9/10/11/17/70/82/148/180/219, 9/10/11/35/70/82/180/200/214, 9/10/11/36/38/65/70/82/180/200/214/229,

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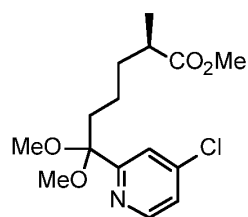
10. The engineered carboxyesterase of Claim 1, wherein said polypeptide sequence is selected from the even-numbered sequence identifiers of: SEQ ID NOs: 4-536.

11. The engineered carboxyesterase of Claim 1, wherein said engineered carboxyesterase comprises at least one improved property compared to the wild-type *Xanthomonas campestris* carboxyesterase of SEQ ID NO:2.

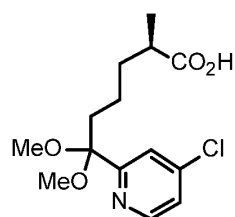
12. The engineered carboxyesterase of Claim 11, wherein said improved property is selected from: improved hydrolysis activity, solvent tolerance, thermostability, pH stability, enantioselectivity, regioselectivity, stereoselectivity, substrate scope, and/or reduced substrate or product inhibition, and reduced toxicity to bacterial host cells producing said engineered carboxyesterase.

13. The engineered carboxyesterase of Claim 12, wherein said bacterial host cells comprise *E. coli*.

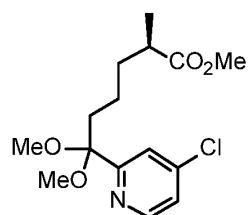
14. The engineered carboxyesterase of Claim 1, wherein said engineered carboxyesterase exhibits greater activity than wild-type *Xanthomonas campestris* carboxyesterase in the conversion of compound (2a)



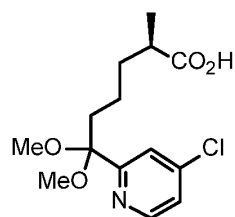
to compound (3a)



15. The engineered carboxyesterase of Claim 1, wherein said engineered carboxyesterase exhibits greater enantioselectivity than wild-type *Xanthomonas campestris* carboxyesterase in the conversion of compound (2a)



to compound (3a)



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

17. The engineered carboxyesterase of Claim 1, wherein said engineered carboxyesterase is immobilized.
18. A composition comprising at least one engineered carboxyesterase of Claim 1.
19. A polynucleotide sequence encoding at least one engineered carboxyesterase of any of Claims 1-18.
20. A polynucleotide sequence encoding at least one engineered carboxyesterase comprising a polypeptide sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 2 or a functional fragment thereof, wherein said engineered carboxyesterase comprises at least one substitution or substitution set in said polypeptide sequence, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2.
21. A polynucleotide sequence encoding at least one engineered carboxyesterase or a functional fragment thereof, said polynucleotide sequence comprising at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a sequence selected from the odd-numbered sequence identifiers of: SEQ ID NOs: 3-535.
22. The polynucleotide sequence of any of Claims 19-21, wherein said polynucleotide sequence is operably linked to a control sequence.
23. The polynucleotide sequence of any of Claims 19-22, wherein said polynucleotide sequence is codon optimized.
24. An expression vector comprising at least one polynucleotide sequence of any of Claims 19-23.
25. A host cell comprising at least one expression vector of Claim 24.
26. A host cell comprising at least one polynucleotide sequence of any of Claims 19-23.
27. The host cell of Claims 25 or 26, wherein said host cell is *E. coli*.

28. A method of producing an engineered carboxyesterase in a host cell, comprising culturing the host cell of any of Claims 25-27, under suitable conditions, such that at least one engineered carboxyesterase is produced.

29. The method of Claim 28, further comprising recovering at least one engineered carboxyesterase from the culture and/or host cell.

30. The method of Claim 29, further comprising the step of purifying said at least one engineered carboxyesterase.