[0006] The present invention further provides isolated recombinant polynucleotide sequences encoding the recombinant cytochrome P450-BM3 polypeptide variants provided herein. In some embodiments, the isolated recombinant polynucleotide sequence comprises SEQ ID NO:1, 3, 5, 7, 9, 11, 13, or 15.

[0007] The present invention also provides expression vectors comprising at least one polynucleotide sequence provided herein. In some additional embodiments, the vector comprises at least one polynucleotide sequence that is operably linked with at least one regulatory sequence suitable for expression of the polynucleotide sequence in a suitable host cell. In some embodiments, the host cell is a prokaryotic or eukaryotic cell. In some additional embodiments, the host cell is a prokaryotic cell. In some further embodiments, the host cell is *E. coli*. The present invention also provides host cells comprising the vectors provided herein.

[0008] The present invention also provides methods for producing at least one recombinant cytochrome P450-BM3 variant comprising culturing the host cell provided herein under conditions such that at least one of the recombinant cytochrome P450-BM3 variants provided herein is produced by the host cell. In some additional embodiments, the methods further comprise the step of recovering at least one recombinant cytochrome P450 variant.

[0008A] The present invention also provides a recombinant cytochrome P450-BM3 variant having at least 80% sequence identity to a polypeptide sequence comprising the sequence set forth in SEQ ID NO:2.

[0008B] The present invention also provides a recombinant cytochrome P450-BM3 variant having at least 80% sequence identity to a polypeptide sequence comprising the sequence set forth in SEQ ID NO:2, wherein the variant has at least 90% sequence identity to a polypeptide sequence comprising the sequence set forth in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, or 16.

[0008C] The present invention also provides a recombinant cytochrome P450-BM3 variant having at least 80% sequence identity to a polypeptide sequence comprising the sequence set forth in SEQ ID NO:2, wherein the variant has at least 90% sequence identity to a polypeptide sequence comprising the sequence set forth in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, or 16, and wherein the variant has a polypeptide sequence comprising the sequence selected from the group comprising:

SEQ ID NO:2 comprising substitutions V51S; D118R; L219Y; K236H; P351T; S353T; N579T; T582A; E624D; A632S; D727S; and Q931K;

SEQ ID NO:2 comprising substitutions V51S; D118R; T179V; L219Y; P351T; N579T; T582A; E624D; D727S;

SEQ ID NO:2 comprising substitutions D118R; L219Y; K236H; S353T; N579T; T582A; E624D; D727S;

SEQ ID NO:4 comprising substitutions C48S; I95P; G115R; L216Y; D232H; M491A; N574T; and D722S;

SEQ ID NO:4 comprising substitutions K32R; C48S; I95P; L216Y; D232H; E349T; M491A; N574T; T577A; E619D; and D722S;

SEQ ID NO:6 comprising substitutions K32R; C48S; Y52F; G115R; L216Y; D232H; E349K; M491A; T577A; E619D; A627S; and D722S;

SEQ ID NO:6 comprising substitutions K32R; C48S; Y52F; I95P; Q111R; G115R; I176V; L216Y; D232H; E349T; M491A; T577A; E619D; and D722S;

SEQ ID NO:8 comprising substitutions Y52F; D232H; E349K; E619D; D722S; R762H;

SEQ ID NO:8 comprising substitutions K32R; Y52F; K95P; Q111R; G115R; I176V; L216Y; D232H; P347T; E349T; M491A; T577A; E619D; and D722S;

SEQ ID NO:8 comprising substitutions K95P; Q111R; G115R; I176V; L216Y; E349T; M491A; T577A; E619D; and D722S;

SEQ ID NO:10 comprising substitutions K32R; C48S; Y52F; I95P; L216Y; D232H; E349K; M491A; N574T; and D722S;

SEQ ID NO:10 comprising substitutions K32R; C48S; Y52F; I95P; Q111R; L216Y; D232H; P347T; E349K; M491A; N574T; E619D; and D722S;

SEQ ID NO:10 comprising substitutions C48S; I95P; G115R; L216Y; S231R; D232H; M491A; N574T; T577A; E619D; and D722S;

SEQ ID NO:12 comprising substitutions K32R; C48S;Y52F; I95P;Q111R;G115R; D232H; M491A; T577A; A627S; D722S;

SEQ ID NO:12 comprising substitutions K32R; C48S; Y52F; I95P; G115R; D232H; M491A; N574T; T577A; E619D; A627S; and D722S;

SEQ ID NO:14 comprising substitutions Y52F; K95P; G115R; T176V; L216Y; D232H; E349T; M491A; T577A; and E619D;

SEQ ID NO:14 comprising substitutions Y52F; G115R; T176V; L216Y; D232H; P347T; E349T; T577A; D722S;

SEQ ID NO:16 comprising substitutions K32R; C48S; Y52F; I95P; I176V; L216Y; D232H; E349K; M491A; N574T; E619D; D722S; A767T; and

SEQ ID NO:16 comprising substitutions C48S; Y52F; I95P; Q111R; I176V; L216Y; D232H; P347T; M491A; N574T; and D722S.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] Figure 1 provides the structures of the substrates used in the screening methods described herein. Diclofenac was used for HTP screening to detect/rank beneficial diversity. The remaining substrates were used to validate that the evolved BM3 variants were active on substrates that were not used for HTP screening.

[0010] Figure 2 provides a graphical summary of the results obtained for the MCYP-1.2-A12 lineage.

[0011] Figure 3 provides a graphical summary of the results obtained for the MCYP-1.2.-A05 lineage.

DESCRIPTION OF THE INVENTION

[0012] The present invention provides improved P450-BM3 variants with improved activity. In some embodiments, the P450-BM3 variants exhibit improved activity over a wide range of substrates. P450-BM3 enzymes exhibit the highest rate of catalysis amongst P450 monooxygenases due to the efficient electron transfer between the fused reductase and heme domains (See e.g., Noble et al., Biochem. J., 339:371-379 [1999]; and Munro et al., Eur. J. Biochem., 239:403-409 [2009]). Thus, P450-BM3 is a highly desirable enzyme for the manipulation of biotechnological processes (See e.g., Sawayama et al., Chem., 15:11723-11729 [2009]; Otey et al., Biotechnol. Bioeng., 93:494-499 [2006]; Damsten et al., Biol. Interact., 171:96-107 [2008]; and Di Nardo and Gilardi, Int. J. Mol. Sci., 13:15901-15924). However, there still remains a need in the art for P450 enzymes that exhibit

The Claims defining the Invention are as follows:

1. A recombinant cytochrome P450-BM3 variant having at least 80% sequence identity to a polypeptide sequence comprising the sequence set forth in SEQ ID NO:2.

2. The recombinant cytochrome P450-BM3 variant of Claim 1, wherein the variant has at least 90% sequence identity to a polypeptide sequence comprising the sequence set forth in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, or 16.

3. The recombinant cytochrome P450-BM3 variant of Claim 1 or Claim 2, wherein the variant has a polypeptide sequence comprising the sequence selected from the group comprising:

SEQ ID NO:2 comprising substitutions V51S; D118R; L219Y; K236H; P351T; S353T; N579T; T582A; E624D; A632S; D727S; and Q931K;

SEQ ID NO:2 comprising substitutions V51S; D118R; T179V; L219Y; P351T; N579T; T582A; E624D; D727S;

SEQ ID NO:2 comprising substitutions D118R; L219Y; K236H; S353T; N579T; T582A; E624D; D727S;

SEQ ID NO:4 comprising substitutions C48S; I95P; G115R; L216Y; D232H; M491A; N574T; and D722S;

SEQ ID NO:4 comprising substitutions K32R; C48S; I95P; L216Y; D232H; E349T; M491A; N574T; T577A; E619D; and D722S;

SEQ ID NO:6 comprising substitutions K32R; C48S; Y52F; G115R; L216Y; D232H; E349K; M491A; T577A; E619D; A627S; and D722S;

SEQ ID NO:6 comprising substitutions K32R; C48S; Y52F; I95P; Q111R; G115R; I176V; L216Y; D232H; E349T; M491A; T577A; E619D; and D722S;

SEQ ID NO:8 comprising substitutions Y52F; D232H; E349K; E619D; D722S; R762H;

SEQ ID NO:8 comprising substitutions K32R; Y52F; K95P; Q111R; G115R; I176V; L216Y; D232H; P347T; E349T; M491A; T577A; E619D; and D722S;

SEQ ID NO:8 comprising substitutions K95P; Q111R; G115R; I176V; L216Y; E349T; M491A; T577A; E619D; and D722S;

SEQ ID NO:10 comprising substitutions K32R; C48S; Y52F; I95P; L216Y; D232H; E349K; M491A; N574T; and D722S;

SEQ ID NO:10 comprising substitutions K32R; C48S; Y52F; I95P; Q111R; L216Y; D232H; P347T; E349K; M491A; N574T; E619D; and D722S;

SEQ ID NO:10 comprising substitutions C48S; I95P; G115R; L216Y; S231R; D232H; M491A; N574T; T577A; E619D; and D722S;

SEQ ID NO:12 comprising substitutions K32R; C48S; Y52F; I95P; Q111R; G115R; D232H; M491A; T577A; A627S; D722S;

SEQ ID NO:12 comprising substitutions K32R; C48S; Y52F; I95P; G115R; D232H; M491A; N574T; T577A; E619D; A627S; and D722S;

SEQ ID NO:14 comprising substitutions Y52F; K95P; G115R; T176V; L216Y; D232H; E349T; M491A; T577A; and E619D;

SEQ ID NO:14 comprising substitutions Y52F; G115R; T176V; L216Y; D232H; P347T; E349T; T577A; D722S;

SEQ ID NO:16 comprising substitutions K32R; C48S; Y52F; I95P; I176V; L216Y; D232H; E349K; M491A; N574T; E619D; D722S; A767T; and

SEQ ID NO:16 comprising substitutions C48S; Y52F; I95P; Q111R; I176V; L216Y; D232H; P347T; M491A; N574T; and D722S.

4. The recombinant cytochrome P450-BM3 variant of any one of Claims 1 to 3, wherein said variant oxidizes at least three organic substrates.

5. The recombinant cytochrome P450-BM3 variant of Claim 4, wherein said organic substrate is selected from the group comprising loratadine, imatinib, geftinib, and diclofenac.

6. The recombinant cytochrome P450-BM3 variant of any one of Claims 1 to 5, wherein the variant exhibits improved oxidizing activity relative to the oxidizing activity exhibited by SEQ ID NO:2, with respect to an organic substrate selected from the group comprising loratadine, imatinib, geftinib, and diclofenac.

7. The recombinant cytochrome P450-BM3 variant of Claim 6, wherein the variant exhibits a Fold Improvement Over Positive Control (FIOPC) of at least 3.0.

8. A recombinant polynucleotide sequence encoding the recombinant cytochrome P450-BM3 variant of any one of Claims 1 to 7.

9. The recombinant polynucleotide sequence of Claim 8, wherein said sequence comprises SEQ ID NO:1, 3, 5, 7, 9, 11, 13, or 15.

10. An expression vector comprising the polynucleotide sequence of Claim 8 or 9.

11. The vector of Claim 10, wherein said polynucleotide sequence is operably linked with regulatory sequences suitable for expression of said polynucleotide sequence in a suitable non-human host cell.

12. The vector of Claim 10 or Claim 11, wherein said non-human host cell is a prokaryotic or eukaryotic cell.

13. The vector of Claim 12, wherein said non-human host cell is a prokaryotic cell.

14. The vector of Claim 12 or 13, wherein said non-human host cell is *E. coli*.

15. A non-human host cell comprising the vector of Claim 10 or 11.

16. A method for producing at least one recombinant cytochrome P450-BM3 variant comprising culturing the non-human host cell of Claim 15 under conditions such that the recombinant cytochrome P450-BM3 variant of any of Claims 1 to 5 is produced by said non-human host cell.

17. The method of Claim 16, further comprising the step of recovering said at least one recombinant cytochrome P450 variant.