SUMMARY

[0007] In one example, the present disclosure refers to a modified galactose oxidase comprising a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 6, wherein SEQ ID NO: 6 is a polypeptide sequence of galactose oxidase variant M3-5, wherein the galactose oxidase variant M3-5 comprises mutations of S33P, M93V, G218E, W313F, R353M, Q429T, V517A, and N558D relative to the wildtype galactose oxidase from *Fusarium graminearum* (SEQ ID NO: 4), and wherein the polypeptide sequence of the modified galactose oxidase further comprises at least one mutation at one or more positions selected from the group consisting of 24, 26, 28, 45, 49, 65, 69, 104, 114, 117, 153, 157, 172, 173, 175, 193, 194, 195, 197, 198, 202, 214, 217, 218, 220, 224, 226, 239, 240, 250, 259, 265, 268, 269, 284, 294, 313, 314, 325, 341, 346, 348, 352, 353, 356, 389, 401, 429, 447, 485, 487, 537, 538, 546, 559, 576, 599, and 662, wherein the position of the mutation is numbered with reference to SEQ ID NO: 6.

[0007a] In one aspect, the present disclosure refers to a modified galactose oxidase comprising a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 6, wherein SEQ ID NO: 6 is a polypeptide sequence of galactose oxidase variant M3-5, wherein the galactose oxidase variant M3-5 comprises mutations of S33P, M93V, G218E, W313F, R353M, Q429T, V517A, and N558D relative to the wildtype galactose oxidase from F*usarium graminearum* (SEQ ID NO: 4), and wherein the polypeptide sequence of the modified galactose oxidase further comprises at least one mutation at one or more positions selected from the group consisting of 24, 26, 28, 45, 49, 65, 69, 104, 114, 117, 153, 157, 172, 173, 175, 193, 194, 195, 197, 198, 202, 214, 217, 218, 220, 224, 226, 239, 240, 250, 259, 265, 268, 269, 284, 294, 313, 314, 325, 341, 346, 348, 352, 353, 356, 389, 401, 429, 447, 485, 487, 537, 538, 546, 559, 576, 599, and 662, wherein the position of the mutation is numbered with reference to SEQ ID NO: 6, wherein the mutation is selected from the group consisting of:

- M353F;
- S314C;
- L537G;
- F217V;
- F217P;
- F217Q;
- F217N;
- F217S;
- N268W;
- E218P;
- F217I;
- F217T;
- F217L;
- F217E;
- F217Y;
- F217A;
- F217C;
- F217D;
- F217G;
- F217H;
- F217K;
- F217M;
- F217R;
- a combination of F217P and E218P;

- a combination of F217P and L537G;
- a combination of F217P, E218P and L537G;
- a combination of F217V and E218P;
- a combination of F217V and L537G;
- a combination of F217V, E218P and L537G;
- a combination of E218P and L537G;
- a combination of F217A and N268W;
- a combination of F217A, N268W and M353F;
- a combination of F217A, N268W and S314C;
- a combination of F217G and N268W;
- a combination of F217G, N268W and M353F;
- a combination of F217A, N268W and A195S;
- a combination of F217A, N268W and V193T;
- a combination of F217A, N268W and N259T;
- a combination of F217A, N268W and Y325F;
- a combination of F217A, N268W and G294A;
- a combination of F217A, N268W and A195T;
- a combination of F217A, N268W and Y352T;
- a combination of F217A, N268W and N341W;
- a combination of F217A, N268W and A197C;
- a combination of F217A, N268W and R240G;
- a combination of F217A, N268W and A198I;
- a combination of F217A, N268W and K348S;
- a combination of F217A, N268W and T202D;
- a combination of F217A, N268W and T202Q;
- a combination of F217A, N268W and T202I;
- a combination of F217A, N268W and T202K;
- a combination of F217A, N268W and P194C;
- a combination of F217A, N268W and A346L;
- a combination of F217A, N268W and K389I;
- a combination of F217A, N268W and Y352W;
- a combination of F217A, N268W and C538K;
- a combination of F217A, N268W and C538R;
- a combination of F217A, N268W and F250E;
- a combination of F217A, N268W and F250Y;
- a combination of F217A, N268W and N356K;
- a combination of F217A, N268W and N356R;
- a combination of F217A, N268W and N356W;
- a combination of F217A, N268W and F487R;
- a combination of F217A, N268W and T429W;
- a combination of F217A, N268W and E218K;
- a combination of F217A, N268W and F313R;
- a combination of F217A, N268W and I284Y;
- a combination of F217A, N268W and A24H;
- a combination of F217A, N268W and I28R;
- a combination of F217A, N268W and I28M;
- a combination of F217A, N268W and S576D;
- a combination of F217A, N268W and I284S;
- a combination of F217A, N268W and F65P;
- a combination of F217A, N268W and T153R;

- a combination of F217A, N268W and A197G;
- a combination of F217A, N268W and T265S;
- a combination of F217A, N268W and S220T;
- a combination of F217A, N268W and N214S;
- a combination of F217A, N268W and I485A;
- a combination of F217A, N268W and N214R;
- a combination of F217A, N268W and D269N;
- a combination of F217A, N268W and I485R;
- a combination of F217A, N268W and I485K;
- a combination of F217A, N268W and D239E;
- a combination of F217A, N268W and T117D;
- a combination of F217A, N268W and S114R;
- a combination of F217A, N268W and S559P;
- a combination of F217A, N268W and S114N;
- a combination of F217A, N268W and Q662P;
- a combination of F217A, N268W and S173R;
- a combination of F217A, N268W and D239P;
- a combination of F217A, N268W and S172E;
- a combination of F217A, N268W and Q662D;
- a combination of F217A, N268W and S173K;
- a combination of F217A, N268W and A195V;
- a combination of F217A, N268W and E218F;
- a combination of F217A, N268W and L226Q;
- a combination of F217A, N268W and I224W;
- a combination of F217A, N268W and A26R;
- a combination of F217A, N268W and I28F;
- a combination of F217A, N268W and E218Y;
- a combination of F217A, N268W and L226V;
- a combination of F217A, N268W and D239Q;
- a combination of F217A, N268W and F546M;
- a combination of F217A, N268W and A24N;
- a combination of F217A, N268W and A26H;
- a combination of F217A, N268W and Y599M;
- a combination of F217A, N268W and G157N;
- a combination of F217A, N268W and Q45D;
- a combination of F217A, N268W and Q45E;
- a combination of F217A, N268W and Q45N;
- a combination of F217A, N268W and E49K;
- a combination of F217A, N268W and N69D;
- a combination of F217A, N268W and N69E;
- a combination of F217A, N268W and N69F;
- a combination of F217A, N268W and N69H;
- a combination of F217A, N268W and N69L;
- a combination of F217A, N268W and N69M;
- a combination of F217A, N268W and N69T;
- a combination of F217A, N268W and N69W;
- a combination of F217A, N268W and N69Y;
- a combination of F217A, N268W and W104G;
- a combination of F217A, N268W and W104N;
- a combination of F217A, N268W and T175E;

- a combination of F217A, N268W and T175H;
- a combination of F217A, N268W and T175I;
- a combination of F217A, N268W and T175K;
- a combination of F217A, N268W and A401D;
- a combination of F217A, N268W, A195T and Y352W;
- a combination of F217A, N268W, N341W and Y352T;
- a combination of F217A, N268W, A198I and N259T;
- a combination of F217A, N268W, A195S and N341W;
- a combination of F217A, N268W, T153K and A195S;
- a combination of F217A, N268W, I28R and K389I;
- a combination of F217A, N268W, T153R and A195T;
- a combination of F217A, N268W, I28R, T153R, A198I and N341W;
- a combination of F217A, N268W, A195T, N259T and N341W;
- a combination of F217A, N268W, I28R, A195T, N259T and K389I;
- a combination of F217A, N268W, I28R, N259T and N341W;
- a combination of F217A, N268W, I28R, A195S and K389I;
- a combination of F217A, N268W, A195T and N341W;
- a combination of F217A, N268W, I28R, A195T, N341W and K389I;
- a combination of F217A, N268W, I28R, A195T, N259T and T447A;
- a combination of F217A, N268W, I28R, A195S, N259T and N341W;
- a combination of F217A, N268W, I28R, A195T, N259T, N341W and K389I; and
- a combination of F217A, N268W, T153R and N341W.

[0008] In one aspect, the present disclosure refers to a polynucleotide sequence encoding the modified galactose oxidase disclosed herein.

[0009] In one aspect, the present disclosure refers to an expression vector comprising the polynucleotide sequence disclosed herein.

[0010] In one aspect, the present disclosure refers to a host cell comprising the polynucleotide sequence disclosed herein or the expression vector disclosed herein.

[0011] In one aspect, the present disclosure refers to a method of producing a modified galactose oxidase, comprising culturing the host cell disclosed herein under suitable culture conditions such that the modified galactose oxidase is produced.

[0012] In one aspect, the present disclosure refers to a method of producing a ketone from a secondary alcohol, comprising contacting the secondary alcohol with the modified galactose oxidase disclosed herein under suitable conditions for an oxidation reaction.

[0013] In one aspect, the present disclosure refers to a kit for use in the method disclosed herein, wherein the kit comprises the modified galactose oxidase disclosed herein.

BRIEF DESCRIPTION OF DRAWINGS

[0014] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which: