Claims

What is claimed is:

1. A cell-based method of identifying one or more enzyme variants that produces an increased level of a ketone as compared to a reference enzyme comprising the steps of:
   (a) culturing, in a culture medium, one or more cells transformed with an expression vector comprising a polynucleotide sequence encoding one or more enzyme variants;
   (b) incubating the one or more cells with para-methoxy-2-amino benzamidoxime (PMA);
   (c) measuring the level of fluorescence signal emitted by the one or more cells;
   (d) comparing the level of fluorescence signal from step c) with a fluorescence signal measured in a cell transformed with an expression vector comprising a polynucleotide sequence encoding the reference enzyme; and
   (e) identifying the one or more enzyme variants when the level of fluorescence signal measured in the cell is increased compared to the reference enzyme.
2. The cell-based method according to claim 1, wherein the culture medium is R-media.
3. The cell-based method according to any one of claims 1 to 2 wherein the polynucleotide sequence encoding the one or more enzyme variants and the polynucleotide sequence encoding the reference enzyme are operably linked to an inducible promoter.
4. The cell-based method according to any one of claims 1 to 3, wherein the culture medium is supplemented with an inducer, optionally wherein the inducer is supplemented at the beginning of the culture process or the inducer is supplemented to the culture medium when the cells are at a predetermined cell density (OD600), optionally wherein the cells are at an OD600 of between about 0.05 to 1.
5. The cell-based method according to claim 4, wherein the inducer is isopropyl β-D-1-thiogalactopyranoside (IPTG) or lactose.
6. The cell-based method according to claim 5, wherein the total concentration of lactose is between about 10 – 50 mM, optionally wherein the total concentration of lactose is about 30 mM.
7. The cell-based method according to claim 1, wherein the concentration of PMA is between about 5 mM – 15 mM, optionally wherein the concentration of PMA is about 10 mM.

8. The cell-based method according to any one of claims 1 to 7, wherein the PMA is incubated with the cells for a duration of about 5 minutes to about 3 hours.

9. The cell-based method according to claim 8, wherein the PMA is incubated with the cells for about 10 minutes.

10. The cell-based method according to any one of claims 1 to 9, wherein the ketone is selected from the group consisting of a carotenoid, an apocarotenoid, a terpenoid, a triterpenoid and a norisoprenoid, optionally wherein the ketone is an apocarotenoid.

11. The cell-based method according to claim 10, wherein the norisoprenoid is selected from the group consisting of β-ionone, 6-methyl-5-hepten-2-one (MHO), irone, α-ionone and psi-ionone, optionally wherein the apocarotenoid is selected from the group consisting of β-ionone, 6-methyl-5-hepten-2-one (MHO), α-ionone and psi-ionone, optionally wherein the apocarotenoid is β-ionone.

12. The cell-based method according to any one of claims 1 to 11, wherein the one or more cells is a bacterial cell.

13. The cell-based method according to claim 12, wherein the bacterial cell is an *Escherichia coli* cell.

14. The cell-based method according to any one of claims 1 to 13, wherein the one or more enzyme variants is generated by mutation of the reference enzyme, optionally when the mutation is an amino acid mutation at one or more active binding sites, and/or one or more sites involved in accessibility to the one or more active binding sites.

15. The cell-based method according to any one of claims 1 to 14, wherein the reference enzyme is a wild type enzyme or an enzyme with known production yield of the ketone.

16. The cell-based method according to any one of claims 1 to 15, wherein the enzyme variant is a CCD1 variant.

17. A method for identifying one or more enzyme variants that produces an increased level of a ketone as compared to a reference enzyme comprising:

(a) purifying and crystalizing the reference enzyme;

(b) identifying one or more active binding sites and/or one or more sites involved in accessibility to the one or more active binding sites;

(c) introducing one or more amino acid mutations at the active binding sites and/or accessibility sites to generate the one or more enzyme variants;

(d) subjecting the one or more enzyme variants from step c) to the cell-based method of any one of claims 1-16 to identify the one or more enzyme variants that produces the increased level of ketone.

18. The method according to claim 17, wherein the one or more enzyme variants identified has an increased binding affinity for a substrate and/or increased catalytic activity compared to the reference enzyme.