Claims

CLAIMS

1. A method for producing a recombinant polypeptide of interest in a microbial host cell, comprising the steps of
   (a) introducing a polynucleotide encoding the polypeptide of interest into a microbial host cell which has been modified such thatcomprising a recombinant polynucleotide expressing an enzymatic activity selected from the group consisting of ketol-acid reductoisomerase (NADP(+)) activity (EC 1.1.1.86), acetohydroxyacid synthase activity (EC 2.2.1.6), aspartate kinase activity (EC 2.7.2.4), homoserine dehydrogenase activity (EC 1.1.1.3), and L-threonine dehydratase (EC 4.3.1.19) is modulated in said microbial host cell as compared to the enzymatic activity in an unmodified microbial host cell,), and
   (b) expressing said polypeptide of interest in said microbial host cell.

2. The method of claim 1, wherein the produced polypeptide of interest shows lower misincorporation of non-canonical branched-chain amino acids as compared to a polypeptide which has been produced by expression in an unmodified microbial host cell.

3. The method of any one of claims 1 and 2, wherein the method further comprises the isolation of the polypeptide from the cell, and the purification of the polypeptide.

4. The method of claim 3, wherein the purification comprises the enrichment of polypeptides which do not comprise non-canonical branched-chain amino acids.

5. The method of claims 1 and 4, wherein said enzymatic activity is increased by introducing and expressing a polynucleotide encoding a polypeptide having said enzymatic activity in said microbial hostin said host cell as compared to a control cell.

6. The method of any one of claim 5, wherein said microbial host cell does not express an endogenous polypeptide having said enzymatic activity.

7. The method of any one of claims 1 to 6, wherein the polypeptide of interest is a therapeutic peptide or polypeptide.

8. The method of any one of claims 1 to 7, wherein the polynucleotide encoding the polypeptide of interest and/or the polynucleotide encoding the polypeptide having said enzymatic activity is operably linked to an inducible promoter.

9. The method of any one of claims 1 to 8, wherein said microbial host cell is an *Escherichia coli* cell.

10. A method for reducing misincorporation of at least one non-canonical branched-chain amino acid into a recombinant polypeptide of interest expressed in a microbial host cell, said method comprising
    (a) ~~modulating~~Introducing a recombinant polynucleotide expressing a polypeptide having an enzymatic activity selected from the group consisting of ketol-acid reductoisomerase (NADP(+)) activity (EC 1.1.1.86), acetohydroxyacid synthase activity (EC 2.2.1.6), aspartate kinase activity (EC 2.7.2.4), homoserine dehydrogenase activity (EC 1.1.1.3), and L-threonine dehydratase (EC 4.3.1.19) in the microbial host cell,
    (b) introducing a polynucleotide encoding the polypeptide of interest into said microbial host cell, and
    (a) expressing said polypeptide of interest in said microbial host cell.

11. The method of claim 10, wherein at least one non-canonical branched-chain amino acid is selected from norvaline, norleucine and beta-methylnorleucine.

12. Use of a polypeptide having an enzymatic activity selected from the group consisting of ketol-acid reductoisomerase (NADP(+)) activity (EC 1.1.1.86), acetohydroxyacid synthase activity (EC 2.2.1.6), aspartate kinase activity (EC 2.7.2.4), homoserine dehydrogenase activity (EC 1.1.1.3), and L-threonine dehydratase (EC 4.3.1.19), or of a polynucleotide encoding said polypeptide for reducing misincorporation of at least one non-canonical branched-chain amino acid into a recombinant polypeptide of interest produced in a microbial host cell.

13. Use of a microbial host cell for producing a recombinant polypeptide of interest, wherein the microbial host cell has been modified such that an enzymatic activity selected from the group consisting of ketol-acid reductoisomerase (NADP(+)) activity (EC 1.1.1.86), acetohydroxyacid synthase activity (EC 2.2.1.6), aspartate kinase activity (EC 2.7.2.4),

homoserine dehydrogenase activity (EC 1.1.1.3), and L-threonine dehydratase (EC 4.3.1.19) is modulated in said microbial host cell as compared to the enzymatic activity in an unmodified microbial host cell.

14. The method according to any one of claims 1 to 11, or the use of claims 12 or 13, wherein the enzymatic activity is modulated.

15. The method according to any one of claims 1 to 11 and 14, or the use of claim 12, 13 or 14, wherein the polypeptide of interest is a proinsulin.

16. A microbial host cell comprising

(a) a recombinant polynucleotide encoding a polypeptide of interest, and

(b) a recombinant polynucleotide encoding a polypeptide having an enzymatic activity selected from the group consisting of ketol-acid reductoisomerase (NADP(+)) activity (EC 1.1.1.86), acetohydroxyacid synthase activity (EC 2.2.1.6), aspartate kinase activity (EC 2.7.2.4), homoserine dehydrogenase activity (EC 1.1.1.3), and L-threonine dehydratase (EC 4.3.1.19).

17. A bioreactor comprising the microbial host cell of claim 16.

18. The bioreactor of claim 17, wherein bioreactor has a volume of at least 10 L.

~~18.~~