

Claims

Claim 1: A genetically modified microorganism for comprising the pathway for 1,4-butanediol production in combination with at least one of the following:

- (a) production of 1,4-butanediol from glycerol via the condensation of 3-hydroxypropionaldehyde with formyl-CoA derived from C1 compounds such as methane, methanol, formaldehyde or formate;
- (b) production of glycerol from ethylene glycol via the condensation of glycolaldehyde with formyl-CoA derived from C1 compounds such as methane, methanol, formaldehyde or formate; and
- (c) production of glycerol from C1 compounds such as methane, methanol, formaldehyde or formate through iterative condensation of formyl-CoA with formaldehyde followed by glycolaldehyde.

Claim 2: The genetically modified microorganism of claim 1, further comprising an overexpressed glycerol dehydratase catalyzing the conversion of glycerol to 3-hydroxypropionaldehyde.

Claim 3: The genetically modified microorganism of claim 2, further comprising an overexpressed TPP-dependent enzyme selected from a 2-hydroxyacyl-CoA synthase, a 2-hydroxyacyl-CoA lyase, an oxalyl-CoA decarboxylase or a benzaldehyde lyase for converting 3-hydroxypropionaldehyde and formyl-CoA to 2,4-dihydroxybutyryl-CoA.

Claim 4: The genetically modified microorganism of claim 3, further comprising an overexpressed acyl-CoA reductase catalyzing the conversion of 2,4-dihydroxybutyryl-CoA to 2,4-dihydroxybutanal.

Claim 5: The genetically modified microorganism of claim 4, further comprising an overexpressed alcohol dehydrogenase catalyzing the conversion of 2,4-dihydroxybutanal to 1,2,4-butanetriol.

Claim 6: The genetically modified microorganism of claim 5, further comprising an overexpressed diol dehydratase catalyzing the conversion of 1,2,4-butanetriol to 4-hydroxybutanal.

Claim 7: The genetically modified microorganism of claim 6, further comprising an overexpressed alcohol dehydrogenase catalyzing the conversion of 4-hydroxybutanal to 1,4-butanediol.

Claim 8: The genetically modified microorganism of any one of claims 1-7, further comprising an overexpressed enzyme catalyzing the conversion of methanol to formaldehyde, wherein the enzyme is selected from methanol dehydrogenase or alcohol oxidase.

Claim 9: The genetically modified microorganism of any one of claims 1-8, further comprising an overexpressed enzyme catalyzing the conversion of formaldehyde to formate, selected from the group consisting of:

- (a) a formaldehyde dehydrogenase;
- (b) a S-(hydroxymethyl) glutathione synthase, S-(hydroxymethyl) glutathione dehydrogenase and S-formylglutathione hydrolase;
- (c) a mycothiol-dependent formaldehyde dehydrogenase and a hydrolase;
- (d) a formaldehyde activating enzyme (FAE), a methylene-tetrahydromethanopterin dehydrogenase, a methenyl-tetrahydromethanopterin cyclohydrolase and a formyltransferase/hydrolase complex; and
- (e) a methylene-tetrahydrofolate dehydrogenase, a methenyl-tetrahydrofolate cyclohydrolase and a formyltetrahydrofolate deformylase.

Claim 10: The genetically modified microorganism of any one of claims 1-9, further comprising an overexpressed enzyme selected from the group consisting of:

- (a) an acylating formaldehyde dehydrogenase converting formaldehyde to formyl-CoA;
- (b) a formate kinase converting formate to formyl-phosphate and a phosphate formyltransferase converting formyl-phosphate to formyl-CoA;
- (c) an acyl-CoA transferase converting formate to formyl-CoA; and
- (d) an acyl-CoA synthase converting formate to formyl-CoA.

Claim 11: The genetically modified microorganism of any one of claims 1-10, further comprising at least one of the following:

- (a) an overexpressed alcohol dehydrogenase catalyzing the conversion of ethylene glycol to glycolaldehyde; and
- (b) one or more enzymes used in pathway for the production of glycolaldehyde from C1 compounds such as methane, methanol, formaldehyde or formate.

Claim 12: The genetically modified microorganism of claim 11, the one or more enzymes include an overexpressed TPP-dependent enzyme selected from a 2-hydroxyacyl-CoA synthase, a 2-hydroxyacyl-CoA lyase, an oxalyl-CoA decarboxylase or a benzaldehyde lyase, catalyzing the conversion of glycolaldehyde and formyl-CoA to glyceryl-CoA.

Claim 13: The genetically modified microorganism of claim 12, further comprising an overexpressed acyl-CoA reductase enzyme catalyzing the conversion of glyceryl-CoA to glyceraldehyde.

Claim 14: The genetically modified microorganism of claim 13, further comprising an overexpressed alcohol dehydrogenase catalyzing the conversion of glyceraldehyde to glycerol.

Claim 15: The genetically modified microorganism of claim 14, the one or more enzymes further include an overexpressed TPP-dependent enzyme selected from a 2-hydroxyacyl-CoA synthase, a 2-hydroxyacyl-CoA lyase, an oxalyl-CoA decarboxylase or a benzaldehyde lyase, catalyzing the conversion of formaldehyde and formyl-CoA to glycolyl-CoA.

Claim 16: The genetically modified microorganism of claim 15, further comprising an overexpressed acyl-CoA reductase catalyzing the conversion of glycolyl-CoA to glycolaldehyde.

Claim 17: The genetically modified microorganism of any one of claims 1-16, wherein the microorganism is selected from the group consisting of bacteria, yeast and fungi.

Claim 18: The genetically modified microorganism of any one of claims 1-16, wherein the microorganism is selected from the group consisting of *Escherichia sp.*, *Bacillus sp.*, *Pseudomonas sp.*, *Corynebacterium sp.*, *Zymonas sp.*, *Clostridium sp.*, *Streptococcus sp.*, *Rhodococcus sp.*, *Geobacillus sp.*, *Saccharomyces sp.*, *Pichia sp.*, *Yarrowia sp.*, *Methylorubrum sp.*, *Candida sp.*, *Kluyveromyces sp.*, *Aspergillus sp.*, *Penicillium sp.*, *Rhizopus sp.* and *Trichoderma sp.*.

Claim 19: A method for the production of formyl-CoA comprising one of the following:

- (a) contacting formaldehyde with an acylating formaldehyde dehydrogenase;

- (b) contacting formate with a formate kinase forming formyl-phosphate and contacting formyl-phosphate with a phosphate formyltransferase; and
- (c) contacting formate with an acyl-CoA transferase or an acyl-CoA synthase

Claim 20: A method for the production of glycolyl-CoA comprising contacting formyl-CoA and formaldehyde with a TPP-dependent enzyme selected from 2-hydroxyacyl-CoA synthase, 2-hydroxyacyl-CoA lyase, oxalyl-CoA decarboxylase or benzaldehyde lyase.

Claim 21: A method for the production of glycolaldehyde comprising contacting glycolyl-CoA with an acyl-CoA reductase.

Claim 22: A method for the production of glycolyl-CoA comprising contacting formyl-CoA and glycolaldehyde with a TPP-dependent enzyme selected from 2-hydroxyacyl-CoA synthase, 2-hydroxyacyl-CoA lyase, oxalyl-CoA decarboxylase or benzaldehyde lyase.

Claim 23: A method for the production of glyceraldehyde comprising:

- (a) providing glyceryl-CoA obtained by the method of claim 22; and
- (b) converting glyceryl-CoA to glyceraldehyde by contacting glyceryl-CoA with an acyl-CoA reductase catalyzing the conversion of glyceryl-CoA to glyceraldehyde.

Claim 24: A method for the production of glycerol comprising:

- (a) providing glyceraldehyde obtained by the method of claim 23; and
- (b) converting glyceraldehyde to glycerol by contacting glyceraldehyde with an alcohol dehydrogenase.

Claim 25: A method for the production of 3-hydroxypropionaldehyde comprising:

- (a) providing glycerol obtained by the method of claim 24; and
- (b) converting glycerol to 3-hydroxypropionaldehyde by contacting glycerol with a glycerol dehydratase.

Claim 26: A method for the production of 1,4-butanediol comprising:

- (a) providing 3-hydroxypropionaldehyde obtained by the method of claims 25;
- (b) converting 3-hydroxypropionaldehyde to 2,4-dihydroxybutyryl-CoA by contacting formyl-CoA and 3-hydroxypropionaldehyde with a TPP-dependent enzyme selected from 2-hydroxyacyl-CoA synthase, 2-hydroxyacyl-CoA lyase, oxalyl-CoA decarboxylase or benzaldehyde lyase;

- (c) converting 2,4- dihydroxybutyryl-CoA to 2,4-dihydroxybutanal by contacting dihydroxybutyryl-CoA with acyl-CoA reductase;
- (d) converting 2,4-dihydroxybutanal to 1,2,4-butanetriol by contacting 2,4-dihydroxybutanal with alcohol dehydrogenase;
- (e) converting 1,2,4-butanetriol to 4-hydroxybutanal by contacting 1,2,4-butanetriol with diol dehydratase; and
- (f) converting 4-hydroxybutanal to 1,4-butanediol by contacting 4-hydroxybutanal with alcohol dehydrogenase.