

with other feed stocks such as, for example, glucose, dextrose, plant biomass or syngas, to produce valuable products. Microorganisms have been that metabolize methanol, and in some instances do so via methanol dehydrogenase, and in even fewer instances produce valuable products. Increasing MeDH activity will enable improved use of MeOH, improving MeOH as a sole carbon source, decreasing production costs, decreasing amounts of any more expensive secondary or co-carbon source, e.g. glucose, increasing product yields, and providing faster rate of MeOH use.

Summary

The current disclosure is directed towards fusion proteins that include methanol dehydrogenase activity and at least one other activity that promotes formaldehyde fixation. For example, the fusion can include activities which promote the conversion of methanol to formaldehyde and then from formaldehyde to a ketose phosphate such as hexulose 6-phosphate, or fructose-6-phosphate. Alternatively, for example, the fusion can include activities which promote the conversion of methanol to formaldehyde and then from formaldehyde to dihydroxyacetone (DHA) and glyceraldehyde-3-phosphate (G3P), and then to fructose-6-phosphate. The current disclosure is also directed to engineered cells that express such fusion proteins, as well as engineered cells having a heterologous methanol dehydrogenase in combination with a fusion protein that promotes formaldehyde fixation. The engineered cells can display increased methanol uptake, through increased expression of the methanol dehydrogenase fusion and/or by using active variants, or through increased efficiency of fixation of formaldehyde into ketose phosphate compounds as a result of the fusion. And while this promotes increased formation of formaldehyde, the formaldehyde is more rapidly fixed into ketose phosphate compounds such as hexulose 6-phosphate, or fructose-6-phosphate, or to DHA and G3P, due to the localization of the enzymatic activities because of the fusion proteins. Therefore, undesirable levels of formaldehyde do not build up in the cell and cause adverse effects on growth.

Further, the increased flux and formation of ketose phosphate compounds, or to DHA and G3P, can provide precursors to the glycolysis pathway. In turn, increased glycolysis can provide increases in the pools of subsequent intermediate compounds such as glycerol 3-phosphate, pyruvate, and acetyl-CoA. These intermediate compounds can be used by product pathways for forming desired (target) bioproducts such as butanediols (BDOs) like 1,3-butanediol (1,3-BDO) and 1,4-butanediol (1,4-BDO), 4-hydroxybutyrate (4-HB), butadiene, 6-amino caproic acid (6ACA), hexamethylenediamine (HMDA), adipic acid, crotyl alcohol, methyl vinyl carbinol, 3-buten-1-ol, 1,2-propanediol (propylene glycol), n-propanol, 1,3-

propanediol, etc. Therefore, the fusions can be expressed in cells that naturally have such product pathways, or cells engineered to express the fusion proteins can further be engineered to include target product pathway enzymes that use the increased amounts of intermediate products available when the fusion proteins of the disclosure are expressed.

5 The fusion proteins of the disclosure improve the ability of a microorganism to grow on methanol as a carbon source. The improved growth on methanol using methanol pathway fusions is likely due to several benefits the fusions provide: 1) rapid conversion of formaldehyde, a potentially toxic product of the MeDH reaction, to hexulose-6-phosphate (or alternatively to DHA and G3P) due to the localized presence of fused HPS; 2) increased
10 efficiency of the fusion protein(s) in the methanol pathway reduces the amount of total methanol pathway protein required for efficient methanol utilization; 3) the presence of methanol pathway fusion proteins pushes the reaction in the forward, desired direction with respect to methanol utilization.

 In one aspect, the disclosure provides a fusion protein comprising: (1a) a first region
15 comprising methanol dehydrogenase or an enzymatically active portion thereof, and (1b) a second region comprising 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof; (2a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (2b) a second region comprising 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof; and (2c) a third region comprising methanol
20 dehydrogenase activator or an enzymatically active portion thereof; (3a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, and (3b) a second region comprising 6-phospho-3-hexuloisomerase or an enzymatically active portion thereof; (4a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (4b) a second region comprising 6-phospho-3-hexuloisomerase or an
25 enzymatically active portion thereof; and (4c) a third region comprising methanol dehydrogenase activator or an enzymatically active portion thereof; (5a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (5b) a second region comprising 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof; and (5c) a third region comprising 6-phospho-3-hexuloisomerase or an enzymatically
30 active portion thereof; or (6a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (6b) a second region comprising 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof; (6c) a third region comprising 6-phospho-3-hexuloisomerase or an enzymatically active portion thereof; and (6d) a fourth region comprising methanol dehydrogenase activator or an enzymatically active portion
35 thereof; wherein the fusion protein optionally comprises one or more linker amino acid

sequence(s) positioned between two or more of the first, second, and third regions of the fusion protein; or a dihydroxyacetone (DHA) pathway fusion protein comprising: (7a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, and (7b) a second region comprising DHA synthase (DHAS) or an enzymatically active portion thereof; (8a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (8b) a second region comprising DHA synthase or an enzymatically active portion thereof; and (8c) a third region comprising methanol dehydrogenase activator or an enzymatically active portion thereof; (9a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, and (9b) a second region comprising fructose-6-phosphate aldolase or an enzymatically active portion thereof; (10a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (10b) a second region comprising fructose-6-phosphate aldolase (F6PAld) or an enzymatically active portion thereof; and (10c) a third region comprising methanol dehydrogenase activator or an enzymatically active portion thereof; (11a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (11b) a second region comprising DHA synthase or an enzymatically active portion thereof; and (11c) a third region comprising fructose-6-phosphate aldolase or an enzymatically active portion thereof; or (12a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (12b) a second region comprising DHA synthase or an enzymatically active portion thereof; (12c) a third region comprising fructose-6-phosphate aldolase or an enzymatically active portion thereof; and (12d) a fourth region comprising methanol dehydrogenase activator or an enzymatically active portion thereof; wherein the fusion protein optionally comprises one or more linker amino acid sequence(s) positioned between two or more of the first, second, and third regions of the fusion protein.

In another aspect, the disclosure provides a fusion protein comprising: first and second portions of a methanol dehydrogenase, and (a) one or more of 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof, 6-phospho-3-hexuloisomerase or an enzymatically active portion thereof, or methanol dehydrogenase activator or an enzymatically active portion thereof, positioned between the first and second portions of a methanol dehydrogenase, or (b) one or more of DHA synthase or an enzymatically active portion thereof, fructose-6-phosphate aldolase (F6PAld) or an enzymatically active portion thereof, or methanol dehydrogenase activator or an enzymatically active portion thereof, positioned between the first and second portions of a methanol dehydrogenase.

In yet another aspect, the disclosure provides a nucleic acid encoding the fusion protein as described herein.

In yet another aspect, the disclosure provides an expression construct comprising the nucleic acid as described herein.

In yet another aspect, the disclosure provides an engineered cell comprising the fusion protein as described herein, the nucleic acid as described herein, or the expression construct as described herein.

In yet another aspect, the disclosure provides an engineered cell comprising (i) a fusion protein comprising a first region comprising methanol dehydrogenase and a second region comprising methanol dehydrogenase activator, or enzymatically active portions thereof, and (ii) and a fusion protein comprising a first region comprising 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof; and a second region comprising 6-phospho-3-hexuloisomerase or an enzymatically active portion thereof; or (i) an exogenous methanol dehydrogenase or a fusion protein comprising a first region comprising methanol dehydrogenase and a second region comprising methanol dehydrogenase activator, or enzymatically active portions thereof, and (ii) and a fusion protein comprising a first region comprising DHAS or an enzymatically active portion thereof; and a second region comprising F6P Ald or an enzymatically active portion thereof.

In yet another aspect, the disclosure provides a composition comprising the engineered cell as described herein, or a cell extract thereof.

In yet another aspect, the disclosure provides a method of producing a target product or its intermediate comprising culturing the engineered cell of any of claims 24-35 in a culture medium comprising methanol to produce the target product or an intermediate of the target product; wherein (a) the cell expresses at least (i) methanol dehydrogenase (MeDH) or an enzymatically active portion thereof, (ii) 3-hexulose-6-phosphate dehydrogenase (HPS) or an enzymatically active portion thereof, and (iii) 6-phospho-3-hexuloisomerase (PHI) or an enzymatically active portion thereof, and wherein any two or three of (i)-(iii) are present in the form of a fusion protein as described herein; wherein (b) the cell expresses at least (i) methanol dehydrogenase (MeDH) or an enzymatically active portion thereof, (ii) DHA (dihydroxyacetone) synthase or an enzymatically active portion thereof, and (iii) F6P (fructose-6-phosphate) aldolase or an enzymatically active portion thereof, wherein any two or three of (i)-(iii) are present in the form of a fusion protein as described herein; or wherein (c) the cell expresses at least (i) methanol dehydrogenase (MeDH) or an enzymatically active portion thereof, (ii) DHA (dihydroxyacetone) synthase or an enzymatically active portion thereof, and (iii) DHA kinase or an enzymatically active portion thereof, and optionally (iv) fructose-bisphosphate aldolase or an enzymatically active portion thereof, wherein any two or three of (i)-(iii), or (i), (ii), and (iv) are present in the form of a fusion protein as described herein.

In examples the disclosure provides a fusion protein comprising: (1a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, and (1b) a second region comprising 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof; (2a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (2b) a second region comprising 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof; and (2c) a third region comprising methanol dehydrogenase activator or an enzymatically active portion thereof; (3a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, and (3b) a second region comprising 6-phospho-3-hexuloisomerase or an enzymatically active portion thereof; (4a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (4b) a second region comprising 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof; and (4c) a third region comprising methanol dehydrogenase activator or an enzymatically active portion thereof; (5a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (5b) a second region comprising 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof; and (5c) a third region comprising 6-phospho-3-hexuloisomerase or an enzymatically active portion thereof; or (6a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (6b) a second region comprising 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof; (6c) a third region comprising 6-phospho-3-hexuloisomerase or an enzymatically active portion thereof; and (6d) a fourth region comprising methanol dehydrogenase activator or an enzymatically active portion thereof; wherein the fusion protein optionally comprises one or more linker amino acid sequence(s) positioned between two or more of the first, second, and third regions of the fusion protein.

In an alternative example directed to MeDH with one or more DHA pathway enzyme(s) fusion proteins, the disclosure provides a fusion protein comprising: (7a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof and (7b) a second region comprising DHA synthase or an enzymatically active portion thereof; (8a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (8b) a second region comprising DHA synthase or an enzymatically active portion thereof; and (8c) a third region comprising methanol dehydrogenase activator or an enzymatically active portion thereof; (9a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, and (9b) a second region comprising a F6P aldolase or an enzymatically active portion thereof; (10a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (10b) a second region comprising

DHA synthase or an enzymatically active portion thereof; and (10c) a third region comprising methanol dehydrogenase activator or an enzymatically active portion thereof; (11a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (11b) a second region comprising DHA synthase or an enzymatically active portion thereof; and (11c) a third region comprising F6P aldolase or an enzymatically active portion thereof; or (12a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (12b) a second region comprising DHA synthase or an enzymatically active portion thereof; (12c) a third region comprising F6P aldolase or an enzymatically active portion thereof; and (12d) a fourth region comprising methanol dehydrogenase activator or an enzymatically active portion thereof; wherein the fusion protein optionally comprises one or more linker amino acid sequence(s) positioned between two or more of the first, second, and third regions of the fusion protein

Examples of the disclosure also include a nucleic acid encoding the MeDH-containing fusion protein (1-6) or (7-12), expression constructs comprising the nucleic acid, engineered cells comprising the MeDH-containing fusion protein (1-6) or (7-12), such as engineered cells comprising exogenous nucleic acids that express the fusion protein (1-6) or (7-12), and methods for producing a target product or an intermediate thereof comprising culturing the engineered cell comprising the MeDH-containing fusion protein (1-6) or (7-12).

The disclosure also provides fusion proteins where the methanol dehydrogenase is split into at least two portions separated by at least one regions of 3-hexulose-6-phosphate dehydrogenase, 6-phospho-3-hexuloisomerase, or/and methanol dehydrogenase activator, or alternatively separated by at least one region(s) of DHAS, F6P aldolase, or/and methanol dehydrogenase activator. In this example the disclosure provides a fusion protein comprising first and second portions of a methanol dehydrogenase, and one or more of 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof, 6-phospho-3-hexuloisomerase or an enzymatically active portion thereof, or methanol dehydrogenase activator or an enzymatically active portion thereof, positioned between the first and second portions of a methanol dehydrogenase; or alternatively in this example the disclosure provides a fusion protein comprising first and second portions of a methanol dehydrogenase, and one or more of DHAS or an enzymatically active portion thereof, F6P aldolase or an enzymatically active portion thereof, or methanol dehydrogenase activator or an enzymatically active portion thereof, positioned between the first and second portions of a methanol dehydrogenase. Optionally, the fusion protein includes one or more linker amino acid sequence(s) positioned between two or more of the first, second, and third regions of the fusion protein

Examples of the disclosure also include a nucleic acid encoding the fusion protein with a split MeDH sequence, expression constructs comprising the nucleic acid, engineered cells comprising the fusion protein with a split MeDH sequence, such as engineered cells comprising exogenous nucleic acids that express the fusion protein, and methods for producing a target product or an intermediate thereof comprising culturing the engineered cell comprising the fusion protein with a split MeDH sequence.

Other examples of the disclosure are directed to products made from the target product obtained from methods using the engineered cell with the fusion protein. Exemplary products include polymers made with a target bioproducts, such as polymers made from diol target products combined with diacids.

Description of the Drawings

Figures 1A – 1F are schematic diagrams of fusion proteins with a MeDH region that is at the N-terminal portion of the protein.

Figures 1G-1H are schematic diagrams of fusion proteins with a MeDH region that is at the C-terminal portion of the protein.

Figures 2A - 2H are schematic diagrams of fusion proteins with a MeDH region that is between N- and C-terminal portions of the protein.

Figures 3A-3C are schematic diagrams of fusion proteins with a MeDH region that is split into two portions in the fusion protein.

Figures 4A and 4B are schematic illustrations of HPS-PHI and MeDH-HPS-PHI fusion proteins in a methanol pathway for formation of fructose-6-phosphate.

What is claimed is:

1. A fusion protein comprising:

(1a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, and (1b) a second region comprising 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof;

(2a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (2b) a second region comprising 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof; and (2c) a third region comprising methanol dehydrogenase activator or an enzymatically active portion thereof;

(3a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, and (3b) a second region comprising 6-phospho-3-hexuloisomerase or an enzymatically active portion thereof;

(4a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (4b) a second region comprising 6-phospho-3-hexuloisomerase or an enzymatically active portion thereof; and (4c) a third region comprising methanol dehydrogenase activator or an enzymatically active portion thereof;

(5a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (5b) a second region comprising 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof; and (5c) a third region comprising 6-phospho-3-hexuloisomerase or an enzymatically active portion thereof; or

(6a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (6b) a second region comprising 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof; (6c) a third region comprising 6-phospho-3-hexuloisomerase or an enzymatically active portion thereof; and (6d) a fourth region comprising methanol dehydrogenase activator or an enzymatically active portion thereof;

wherein the fusion protein optionally comprises one or more linker amino acid sequence(s) positioned between two or more of the first, second, and third regions of the fusion protein;

or

a dihydroxyacetone (DHA) pathway fusion protein comprising:

(7a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, and (7b) a second region comprising DHA synthase (DHAS) or an enzymatically active portion thereof;

(8a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (8b) a second region comprising DHA synthase or an enzymatically active portion thereof; and (8c) a third region comprising methanol dehydrogenase activator or an enzymatically active portion thereof;

(9a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, and (9b) a second region comprising fructose-6-phosphate aldolase or an enzymatically active portion thereof;

(10a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (10b) a second region comprising fructose-6-phosphate aldolase (F6PAld) or an enzymatically active portion thereof; and (10c) a third region comprising methanol dehydrogenase activator or an enzymatically active portion thereof;

(11a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (11b) a second region comprising DHA synthase or an enzymatically active portion thereof; and (11c) a third region comprising fructose-6-phosphate aldolase or an enzymatically active portion thereof; or

(12a) a first region comprising methanol dehydrogenase or an enzymatically active portion thereof, (12b) a second region comprising DHA synthase or an enzymatically active portion thereof; (12c) a third region comprising fructose-6-phosphate aldolase or an enzymatically active portion thereof; and (12d) a fourth region comprising methanol dehydrogenase activator or an enzymatically active portion thereof;

wherein the fusion protein optionally comprises one or more linker amino acid sequence(s) positioned between two or more of the first, second, and third regions of the fusion protein.

2. A fusion protein comprising:

first and second portions of a methanol dehydrogenase, and

(a) one or more of 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof, 6-phospho-3-hexuloisomerase or an enzymatically active portion thereof, or methanol dehydrogenase activator or an enzymatically active portion thereof, positioned between the first and second portions of a methanol dehydrogenase, or

(b) one or more of DHA synthase or an enzymatically active portion thereof, fructose-6-phosphate aldolase (F6PAld) or an enzymatically active portion thereof, or methanol dehydrogenase activator or an enzymatically active portion thereof, positioned between the first and second portions of a methanol dehydrogenase.

3. The fusion protein of claim 1 having a structure as follows:

(i) 1a-L¹-1b or 1b-L¹-1a;

(ii) 2a-L¹-2b-L²-2c, 2a-L¹-2c-L²-2b, 2b-L¹-2a-L²-2c, 2b-L¹-2c-L²-2a, 2c-L¹-2a-L²-2b, or 2c-L¹-2b-L²-2a;

5 (iii) 3a-L¹-3b or 3b-L¹-3a;

(iv) 4a-L¹-4b-L²-4c, 4a-L¹-4c-L²-4b, 4b-L¹-4a-L²-4c, 4b-L¹-4c-L²-4a, 4c-L¹-4a-L²-4b, or 4c-L¹-4b-L²-4a;

(v) 5a-L¹-5b-L²-5c, 5a-L¹-5c-L²-5b, 5b-L¹-5a-L²-5c, 5b-L¹-5c-L²-5a, 5c-L¹-5a-L²-5b, or 5c-L¹-5b-L²-5a; or

10 (vi) 6a-L¹-6b-L²-6c-L³-6d, 6a-L¹-6b-L²-6d-L³-6c, 6a-L¹-6c-L²-6b-L³-6d, 6a-L¹-6c-L²-6d-L³-6b, 6a-L¹-6d-L²-6b-L³-6c, 6a-L¹-6d-L²-6c-L³-6b, 6b-L¹-6a-L²-6c-L³-6d, 6b-L¹-6a-L²-6c-L³-6d, 6b-L¹-6c-L²-6a-L³-6d, 6b-L¹-6c-L²-6d-L³-6a, 6b-L¹-6d-L²-6a-L³-6c, 6b-L¹-6d-L²-6c-L³-6a, 6c-L¹-6a-L²-6b-L³-6d, 6c-L¹-6a-L²-6d-L³-6b, 6c-L¹-6b-L²-6a-L³-6d, 6c-L¹-6b-L²-6d-L³-6a, 6c-L¹-6d-L²-6a-L³-6b, 6c-L¹-6d-L²-6b-L³-6a, 6d-L¹-6a-L²-6b-L³-6c, 6d-L¹-6a-L²-6c-L³-6b, 6d-L¹-6b-L²-6a-L³-6c, 6d-L¹-6b-L²-6c-L³-6a, 6d-L¹-6c-L²-6b-L³-6a, and 6d-L¹-6c-L²-6a-L³-6b;

wherein L¹, L², and L³ are independently selected from (-) (a covalent bond) and a linker amino acid sequence

or

the fusion protein of claim 1 having a structure as follows:

20 (i) 7a-L¹-7b or 7b-L¹-7a;

(ii) 8a-L¹-8b-L²-8c, 8a-L¹-8c-L²-8b, 8b-L¹-8a-L²-8c, 8b-L¹-8c-L²-8a, 8c-L¹-8a-L²-8b, or 8c-L¹-8b-L²-8a;

(iii) 9a-L¹-9b or 9b-L¹-9a;

(iv) 10a-L¹-10b-L²-10c, 10a-L¹-10c-L²-10b, 10b-L¹-10a-L²-10c, 10b-L¹-10c-L²-10a, 10c-L¹-10a-L²-10b, or 10c-L¹-10b-L²-10a;

(v) 11a-L¹-11b-L²-11c, 11a-L¹-11c-L²-11b, 11b-L¹-11a-L²-11c, 11b-L¹-11c-L²-11a, 11c-L¹-11a-L²-11b, or 11c-L¹-11b-L²-11a; or

(vi) 12a-L¹-12b-L²-12c-L³-12d, 12a-L¹-12b-L²-12d-L³-12c, 12a-L¹-12c-L²-12b-L³-12d, 12a-L¹-12c-L²-12d-L³-12b, 12a-L¹-12d-L²-12b-L³-12c, 12a-L¹-12d-L²-12c-L³-12b, 12b-L¹-12a-L²-12c-L³-12d, 12b-L¹-12a-L²-12d-L³-12c, 12b-L¹-12c-L²-12a-L³-12d, 12b-L¹-12c-L²-12d-L³-12a, 12b-L¹-12d-L²-12a-L³-12c, 12b-L¹-12d-L²-12c-L³-12a, 12c-L¹-12a-L²-12b-L³-12d, 12c-L¹-12a-L²-12d-L³-12b, 12c-L¹-12b-L²-12a-L³-12d, 12c-L¹-12b-L²-12d-L³-12a, 12c-L¹-12d-L²-12a-L³-12b, 12c-L¹-12d-L²-12b-L³-12a, 12d-L¹-12a-L²-12b-L³-12c, 12d-L¹-12a-L²-12c-L³-12b, 12d-L¹-12b-L²-12a-L³-12c, 12d-L¹-12b-L²-12c-L³-12a, 12d-L¹-12c-L²-12b-L³-12a, and 12d-L¹-12c-L²-12a-L³-12b;

wherein L¹, L², and L³ are independently selected from (-) (a covalent bond) and a linker amino acid sequence.

4. The fusion protein of claim 1, 2 or 3 wherein one or more of the methanol dehydrogenase, methanol dehydrogenase activator, the 3-hexulose-6-phosphate dehydrogenase, the 6-phospho-3-hexuloisomerase, the DHA synthase, the fructose-6-phosphate aldolase or enzymatically active portions thereof, is from bacteria.

5. The fusion protein of claim 4 wherein one or more of the methanol dehydrogenase, the methanol dehydrogenase activator, the 3-hexulose-6-phosphate dehydrogenase, the 6-phospho-3-hexuloisomerase, the DHA synthase, the fructose-6-phosphate aldolase or enzymatically active portions thereof, is from *Bacillus*.

6. The fusion protein of claim 5 wherein one or more of the methanol dehydrogenase, the methanol dehydrogenase activator, the 3-hexulose-6-phosphate dehydrogenase, the 6-phospho-3-hexuloisomerase, the DHA synthase, the fructose-6-phosphate aldolase or enzymatically active portions thereof, is from *Bacillus methanolicus* MGA3 or *Bacillus methanolicus* PB1.

7. The fusion protein of claim 1, 2 or 3 wherein the methanol dehydrogenase (MeDH) or an enzymatically active portion thereof is from *Bacillus methanolicus* MGA3 MeDH (3EIJ77596.1); *Bacillus methanolicus* C1 MeDH (AAA22593.1); *Bacillus methanolicus* PB1 MeDH (EIJ77618.1); *Bacillus methanolicus* PB1 MeDH (EIJ78790.1); *Bacillus methanolicus* MGA3 MeDH (EIJ80770.1); *Bacillus methanolicus* PB1 MeDH (EIJ78397.1); *Bacillus methanolicus* MGA3 MeDH (EIJ83020.1); *Lysinibacillus fusiformis* MeDH (EFI69743.1); *Bacillus coagulans* 36D1 MeDH (YP_004860127.1); *Lysinibacillus sphaericus* MeDH (YP_001699778.1); *Bacillus azotoformans* LMG 9581 MeDH (ZP_11313277.1); *Burkholderia thailandensis* E264 MeDH (ZP_05587334.1); *Cupriavidus necator* N-1 MeDH (YP_004681552.1); uncultured organism MeDH (AGF87161); *Geobacter bemidjiensis* Bem MeDH (YP_002138168.1); *Carboxydotherrmus hydrogenoformans* Z-2901 MeDH (YP_359772.1); *Actinobacillus succinogenes* 130Z MeDH (YP_001343716.1); *Acinetobacter baumannii* Naval-82 MeDH (ZP_16224338.1); *Clostridium pasteurianum* DSM 525 MeDH (AAC45651.1); *Methanosarcina mazei* Tuc01 MeDH (YP_007491369.1); *Desulfovibrio vulgaris* str. 'Miyazaki F' MeDH (YP_002434746); *Desulfovibrio africanus* str. Walvis Bay MeDH (YP_005052855); *Clostridium perfringens* str. 13 MeDH (NP_561852.1); *Vibrio campbellii* ATCC BAA-1116 MeDH (YP_001447544); *Desulfotomaculum reducens* MI-1

MeDH (YP_001113612.1); *Desulfovibrio vulgaris* str. Hildenborough MeDH (YP_011618);
Photobacterium profundum 3TCK MeDH (ZP_01220157.1); *Geobacillus* sp. Y4.1MC1
MeDH (YP_003990729.1); *Desulfovibrio fructosovorans* JJ MeDH (ZP_07335453.1);
Shewanella oneidensis MR-1 MeDH (NP_717107); *Sebaldella termitidis* ATCC 33386 MeDH
5 (YP_003310546.1); *Paenibacillus peoriae* KCTC 3763 MeDH (ZP_10241531.1); *Klebsiella*
pneumoniae subsp. *pneumoniae* MGH 78578 MeDH (YP_001337153.1); *Escherichia coli*
MeDH (YP_026233.1); *Clostridium perfringens* ATCC 13124 MeDH (YP_694908); *Ralstonia*
eutropha H16 MeDH (YP_725376.1); *Thermoanaerobacter* sp. X514 MeDH
(YP_001663549); human gut metagenome MeDH (EKC54576); or *Geobacillus*
10 *thermodenitrificans* NG80-2 MeDH (YP_001126968.1).

8. The fusion protein of claim 1, 2 or 3 wherein the methanol dehydrogenase or an
enzymatically active portion thereof is a polypeptide having 50% or greater, 60% or greater,
70% or greater, 80% or greater, 85% or greater, 90% or greater, 95% or greater, 96% or
15 greater, 97% or greater, 98% or greater, or 99% or greater identity to *Bacillus methanolicus*
MGA3MeDH (3EIJ77596.1) or to any other MeDH according to claim 7.

9. The fusion protein of claim 8 comprising one or more amino acid substitutions that differ
from a native sequence of the methanol dehydrogenase, 3-hexulose-6-phosphate
20 dehydrogenase, 6-phospho-3-hexuloisomerase, the DHA synthase, the fructose-6-phosphate
aldolase or methanol dehydrogenase activator.

10. The fusion protein of claim 1, 2 or 3 wherein the region comprising 3-hexulose-6-
phosphate dehydrogenase or an enzymatically active portion thereof is from *Bacillus*
25 *methanolicus* MGA3 HPS (AAR39392.1); *Bacillus methanolicus* PB1 HPS (EIJ81375.1);
Methylobacillus flagellatus HPS (YP_544362.1); *Methylobacillus flagellatus* HPS (YP
544362.1); *Bacillus subtilis* HPS (NP_388228.1); *Methylophilus methylotrophus* HPS
(WP_018986666.1); or *Methylophilus methylotrophus* ATCC 53528 HPS (WP_018985298.1).

30 11. The fusion protein of claim 1, 2 or 3 wherein the region comprising DHA synthase
(DHAS) or an enzymatically active portion thereof is from *Mycobacter* sp. strain JC1 DSM
3803 (AAG12171.2); *Ogataea parapolymorpha* DL-1 (*Hansenula polymorpha* DL-1)
(EFW95760.1); or *Candida boidinii* (AAC83349.1).

12. The fusion protein of claim 1, 2 or 3 wherein the region comprising fructose-6-phosphate aldolase (F6PAld) or an enzymatically active portion thereof is from *Amycolatopsis methanolica* 239 (AIJ24623.1); *Bacillus methanolicus* MGA3 plasmid pBM19 (WP_003349819.1); *Bacillus methanolicus* PB1 plasmid pBM20 (WP_003352247.1);
- 5 *Geobacillus thermodenitrificans* NG80-2 (WP_008880720.1); *Geobacillus thermodenitrificans* NG80-2 (WP_008880032.1); *Methylophilus methylotrophus* ATCC 53528 (WP_018986971.1); and *Bacillus methanolicus* MGA3 (WP_003346852.1)
13. The fusion protein of claim 1, 2 or 3, wherein 3-hexulose-6-phosphate dehydrogenase or an
- 10 enzymatically active portion thereof is a polypeptide having 50% or greater, 60% or greater, 70% or greater, 80% or greater, 85% or greater, 90% or greater, 95% or greater, 96% or greater, 97% or greater, 98% or greater, or 99% or greater identity to *Bacillus methanolicus* MGA3 HPS (AAR39392.1) or to any other HPS according to claim 10;
- or
- 15 the fusion protein of claim 1, 2 or 3, wherein DHAS or an enzymatically active portion thereof is a polypeptide having 50% or greater, 60% or greater, 70% or greater, 80% or greater, 85% or greater, 90% or greater, 95% or greater, 96% or greater, 97% or greater, 98% or greater, or 99% or greater identity to any one of the DHAS described herein.
- 20 14. The fusion protein of claim 1, 2 or 3, wherein 6-phospho-3-hexuloisomerase or an enzymatically active portion thereof is from *Bacillus methanolicus* MGA3 PHI (AAR39393.1); *Bacillus methanolicus* PB1 PHI (EIJ81376.1); *Mycobacterium gastri* PB1 PHI (BAA90545.1); *Methylobacillus flagellatus* KT PHI (YP_545762.1); *Bacillus subtilis* PHI (NP_388227.1); or *Methylophilus methylotrophus* ATCC 53528 HPS (WP_018985297.1).
- 25
15. The fusion protein of claim 1, 2 or 3, wherein the region comprising 6-phospho-3-hexuloisomerase or an enzymatically active portion thereof is a polypeptide having 50% or greater, 60% or greater, 70% or greater, 80% or greater, 85% or greater, 90% or greater, 95% or greater, 96% or greater, 97% or greater, 98% or greater, or 99% or greater identity to
- 30 *Bacillus methanolicus* MGA3 PHI (AAR39393.1) or to any other PHI according to claim 12;
- or
- the fusion protein of claim 1, 2 or 3, wherein the region comprising F6P Ald or an enzymatically active portion thereof is a polypeptide having 50% or greater, 60% or greater, 70% or greater, 80% or greater, 85% or greater, 90% or greater, 95% or greater, 96% or

greater, 97% or greater, 98% or greater, or 99% or greater identity to any one of the F6P Ald described herein.

16. The fusion protein of claim 1, 2 or 3, wherein the region comprising the methanol dehydrogenase activator or an enzymatically active portion thereof is a polypeptide having 50% or greater, 60% or greater, 70% or greater, 80% or greater, 85% or greater, 90% or greater, 95% or greater, 96% or greater, 97% or greater, 98% or greater, or 99% or greater identity to *Bacillus methanolicus* MGA3 ACT (Q8KP10) or a paralog or ortholog of MGA3ACT.

17. The fusion protein of any one of the previous claims having one linker amino acid sequence.

18. The fusion protein of any of claims 1-17 having two linker amino acid sequences, wherein the two linker amino acid sequences are the same or different.

19. The fusion protein of any one of the previous claims, wherein the linker amino acid sequence comprises one or more amino acids selected from the group consisting of glycine, alanine, serine, and threonine.

20. The fusion protein of any one of the previous claims, wherein the linker amino acid sequence has a length in the range of 1-50 amino acid residues, or 1-20 amino acid residues.

21. The fusion protein of any one of the previous claims, wherein the linker amino acid sequence is $[(\text{GGS})_x(\text{GS})_y(\text{GGS})_z]_n$ where x is 1-6, y is 1-6, z is 0-3, and n is 1-4, preferably where x is 1-3, y is 1-3, z is 1-2, and n is 1-2.

22. A nucleic acid encoding the fusion protein of any of the previous claims.

23. An expression construct comprising the nucleic acid of claim 22.

24. An engineered cell comprising the fusion protein of any one of claims 1-21, the nucleic acid of claim 22, or the expression construct of claim 23.

25. The engineered cell of claim 24 which is a microbial cell.

26. The engineered cell of claim 25 which is a bacterial cell.

27. The engineered cell of claim 26 which is *E. coli*.

5

28. The engineered cell of any one of claims 24-27 further comprising one or more transgene(s) encoding a protein of a metabolic pathway that promotes production of a target product or intermediate thereof, wherein the metabolic pathway uses a compound resulting from methanol consumption by the cell.

10

29. The engineered cell of any one of claims 24-27 wherein expression of the fusion provides an increased amount of reducing equivalents for an increase in a target product and/or for increased fixation of carbon from formaldehyde into a target product.

15

30. The engineered cell of any one of claims 24-27 further comprising a transgene encoding an enzyme to convert the formaldehyde to formate thereby generating reducing equivalents useful to product the target product and/or able to fix carbon of formate into the target product.

20

31. The engineered cell of any one of claims 24-27 capable of producing a greater amount of acetyl CoA in the presence of methanol as compared to a cell that does not express the fusion protein.

32. The engineered cell of any one of claims 24-27 comprising a metabolic pathway capable of producing a target product selected from the group consisting of 1,4-butanediol, 1,3-butanediol, butadiene, succinate, adipate, HMDA, or 6-aminocaproic acid (6ACA).

25

33. An engineered cell comprising (i) a fusion protein comprising a first region comprising methanol dehydrogenase and a second region comprising methanol dehydrogenase activator, or enzymatically active portions thereof, and (ii) and a fusion protein comprising a first region comprising 3-hexulose-6-phosphate dehydrogenase or an enzymatically active portion thereof; and a second region comprising 6-phospho-3-hexuloisomerase or an enzymatically active portion thereof;

30

or

(i) an exogenous methanol dehydrogenase or a fusion protein comprising a first region comprising methanol dehydrogenase and a second region comprising methanol dehydrogenase

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activator, or enzymatically active portions thereof, and (ii) and a fusion protein comprising a first region comprising DHAS or an enzymatically active portion thereof; and a second region comprising F6P Ald or an enzymatically active portion thereof.

5 34. The engineered cell of any one of claims 24-33 comprising any one or more enzymes selected from the group consisting of DHA (dihydroxyacetone) synthase, F6P (fructose-6-phosphate) aldolase, DHA kinase, fructose-bisphosphate aldolase, wherein any one or more of the enzymes is optionally expressed from an exogenous nucleic acid.

10 35. The engineered cell of any one of claims 24-34 comprising any one or more enzymes selected from the group consisting of succinyl-CoA synthetase; CoA-independent succinic semialdehyde dehydrogenase; α -ketoglutarate dehydrogenase; glutamate:succinate semialdehyde transaminase; glutamate decarboxylase; CoA-dependent succinic semialdehyde dehydrogenase; 4-hydroxybutanoate dehydrogenase; α -ketoglutarate decarboxylase; 4-
15 hydroxybutyryl CoA: acetyl-CoA transferase; butyrate kinase; phosphotransbutyrylase; aldehyde dehydrogenase; alcohol dehydrogenase, wherein any one or more of the enzymes is optionally expressed from an exogenous nucleic acid.

20 36. A composition comprising the engineered cell of any of claims 24-35, or a cell extract thereof.

37. The composition of claim 36 wherein the composition is a cell culture composition, optionally comprising a target product or intermediate thereof.

25 38. A method of producing a target product or its intermediate comprising culturing the engineered cell of any of claims 24-35 in a culture medium comprising methanol to produce the target product or an intermediate of the target product;

wherein (a) the cell expresses at least (i) methanol dehydrogenase (MeDH) or an enzymatically active portion thereof, (ii) 3-hexulose-6-phosphate dehydrogenase (HPS) or an
30 enzymatically active portion thereof, and (iii) 6-phospho-3-hexuloisomerase (PHI) or an enzymatically active portion thereof, and wherein any two or three of (i)-(iii) are present in the form of a fusion protein as described herein;

wherein (b) the cell expresses at least (i) methanol dehydrogenase (MeDH) or an enzymatically active portion thereof, (ii) DHA (dihydroxyacetone) synthase or an
35 enzymatically active portion thereof, and (iii) F6P (fructose-6-phosphate) aldolase or an

enzymatically active portion thereof, wherein any two or three of (i)-(iii) are present in the form of a fusion protein as described herein; or

wherein (c) the cell expresses at least (i) methanol dehydrogenase (MeDH) or an enzymatically active portion thereof, (ii) DHA (dihydroxyacetone) synthase or an

- 5 enzymatically active portion thereof, and (iii) DHA kinase or an enzymatically active portion thereof, and optionally (iv) fructose-bisphosphate aldolase or an enzymatically active portion thereof, wherein any two or three of (i)-(iii), or (i), (ii), and (iv) are present in the form of a fusion protein as described herein.

- 10 39. The method of claim 38 wherein the culture medium comprises methanol and a second carbon source.

40. The method of claim 38 or 39 further comprising a step of isolating or purifying the target product or an intermediate of the target product.