

DESCRIPTION

TITLE OF THE INVENTION: PRODUCTION OF 1,4-BUTANEDIOL VIA CONDENSATION OF C1-DERIVED FORMYL-COA AND 3- HYDROXYPROPIONALDEHYDE DERIVED FROM GLYCEROL

TECHNICAL FIELD

[0001] The present disclosure generally relates to a method for producing 1,4-butanediol, in particular, a method for producing 1,4-butanediol via condensation of C1-derived formyl-CoA and 3-hydroxypropionaldehyde. Firstly, the present disclosure provides a method for the production of 1,4-butanediol via condensation of 3-hydroxypropionaldehyde and C1-derived formyl-CoA, wherein 3-hydroxypropionaldehyde is derived from the dehydration of glycerol. Secondly, the present disclosure provides a method for the production of glycerol via condensation of glycolaldehyde and C1-derived formyl-CoA, wherein glycolaldehyde is derived from the oxidation of ethylene glycol. Thirdly, the present disclosure provides a method for producing glycerol via condensation of glycolaldehyde and C1-derived formyl-CoA, wherein glycolaldehyde is produced from the condensation of formaldehyde and C1-derived formyl-CoA. Additionally, this disclosure describes the method for generation of formyl-CoA from C1 compounds such as methane, methanol, formaldehyde or formate. The present disclosure also relates to genetically modified microorganism used for producing 1,4-butanediol.

SUMMARY OF THE INVENTION

[0002] The present disclosure provides the following exemplary embodiments.

[0003] In an aspect, provided herein is a method for the production of 1,4-butanediol from glycerol via the condensation of 3-hydroxypropionaldehyde and formyl-CoA derived from C1 compounds such as methane, methanol, formaldehyde or formate.

[0004] Embodiment 1. A method for the production of 1,4-butanediol from glycerol comprising one or more of the following:

- (a) condensation of 3-hydroxypropionaldehyde and C1-derived formyl-CoA, for example by the method described in any one of Embodiments 2-14;
- (b) production of glycerol from ethylene glycol via condensation of glycolaldehyde with formyl-CoA derived from C1 compounds such as methane, methanol, formaldehyde or formate, for example by the method described in Embodiments 15-36;
- (c) production of glycerol from C1 compounds such as methane, methanol, formaldehyde or formate through iterative condensation of formyl-CoA with formaldehyde (for example by the method described in Embodiments 37-42) followed by the condensation of glycolaldehyde with formyl-CoA (for example by the method described in Embodiments 15-36).

[0005] Embodiment 2. The method of Embodiment 1, comprising generating 3-hydroxypropionaldehyde from glycerol by contacting glycerol with a glycerol dehydratase.

[0006] Embodiment 3. The method of Embodiment 2, wherein the glycerol dehydratase is selected from:

GDHt#	GenBank Accession Number	GDHt#	GenBank Accession Number	
1	Q59476, O08505, Q59475	16	NP_418386	
2	Q8GEZ8, Q8GEZ7	17	ZP_03717880.1	
3	P45514, A8CH91, A8CH95	18	YP_356813.1	
4	Q59470, Q59471, Q59472	19	WP_011471631.1	
5	A0A828U7A4, A0A828U8K0	20	WP_011388658.1	
6	ACI39932	21	ABC25539	
7	ZP_03292457.1	22	ZP_02040253.1	
8	ZP_03706520.1	23	ZP_01962384.1	
9	WP_011721311.1	24	ZP_02701301.1	
10	WP_012199200.1	25	NP_462994	
11	ABW38375	26	WP_011787819.1	
12	WP_011099190.1	27	NP_709751	
13	WP_015943317.1	28	ZP_00789518.1	or
14	ZP_03467192.1	29	ZP_01867938.1	.
15	ZP_02692139.1			

[0007] Embodiment 4. A method for producing 1,4-butanediol comprising:

- (a) providing 3-hydroxypropionaldehyde by the method of Embodiment 2 or 3;
- (b) forming 2,4-dihydroxybutyryl-CoA via condensation of 3-hydroxypropionaldehyde with C1-derived formyl-CoA;

- (c) forming 2,4-dihydroxybutanal from 2,4-dihydroxybutyryl-CoA;
- (d) forming 1,2,4-butanetriol from 2,4-dihydroxybutanal;
- (e) forming 4-hydroxybutanal from 1,2,4-butanetriol; and
- (f) forming 1,4-butanediol from 4-hydroxybutanal.

[0008] Embodiment 5. The method of Embodiment 4, wherein 2,4-dihydroxybutyryl-CoA is produced 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.

[0009] Embodiment 6. The method of Embodiment 5, wherein the TPP-dependent enzyme is 2- 2-hydroxyacyl-CoA synthase or 2-hydroxyacyl-CoA lyase, selected from:

HACS#	GenBank Accession Number	HACS#	GenBank Accession Number	HACS#	GenBank Accession Number
1	XP_012756082.1	47	MXY78649.1	93	TAJ19927.1
2	TMK01573.1	48	MBA01399.1	94	PKN81274.1
3	PYM26381.1	49	MXX31676.1	95	RLT34960.1
4	EEG70177.1	50	MXV80929.1	96	MBT5775398.1
5	MBH80817.1	51	MBI4083577.1	97	TMD99851.1
6	WP_030891887.1	52	MBK6319978.1	98	MSQ12864.1
7	AGK93615.1	53	MBI5948182.1	99	MBL0714078.1
8	MAX57815.1	54	PFG74273.1	100	WP_114297888.1
9	WP_068916287.1	55	WP_158065972.1	101	MAK25262.1
10	WP_062165271.1	56	MBN9492325.1	102	WP_068138361.1
11	MBB43458.1	57	MBK6663287.1	103	RMG94145.1
12	PCJ72347.1	58	MBI2766664.1	104	MBA4180234.1
13	TMQ19149.1	59	HEM18354.1	105	MBM3723043.1
14	MAX11513.1	60	GBD22648.1	106	ABF11225.1
15	HAK63664.1	61	MBF6599205.1	107	TAL98798.1
16	MBG92919.1	62	MXW00101.1	108	NNN20496.1
17	PZC46201.1	63	MYA07641.1	109	MBP1761901.1
18	MBB84818.1	64	REJ76484.1	110	PPQ43247.1
19	OGA51379.1	65	HDY15625.1	111	MSQ25793.1
20	PWB41796.1	66	MBW2231087.1	112	TMK28344.1
21	MAE93843.1	67	NRA08835.1	113	HIB12002.1
22	OGP60024.1	68	NQZ98823.1	114	WP_179589464.1
23	OWB57166.1	69	MBI3918747.1	115	MXY42918.1
24	KXN72624.1	70	MBI2761137.1	116	WP_184156128.1

25	PVU86112.1	71	MBE0608783.1	117	HET53513.1
26	ORZ16580.1	72	MYA54281.1	118	TMK22624.1
27	XP_005644825.1	73	NRA01576.1	119	MXX66290.1
28	KZV27770.1	74	MBW2623123.1	120	GIS94895.1
29	EJY87672.1	75	MBI5615765.1	121	MBN1557905.1
30	HIG47824.1	76	MSR14309.1	122	MSV30368.1
31	TMD03111.1	77	XP_004342722.2	123	MBN2179295.1
32	MBJ56818.1	78	MSP42197.1	124	TDI90456.1
33	WP_095860310.1	79	TDI61101.1	125	OGN76415.1
34	MBL8483477.1	80	MBO0741576.1	126	WP_102074055.1
35	WP_058697592.1	81	MBO0736096.1	127	PZC47999.1
36	WP_130292058.1	82	MBV9828771.1	128	HHH88785.1
37	WP_207956071.1	83	MAW55136.1	129	OLB93949.1
38	WP_132429652.1	84	MBV38827.1	130	PKB76696.1
39	WP_060575023.1	85	TMJ68231.1	131	HED24197.1
40	WP_068796145.1	86	TMJ64557.1	132	WP_066960443.1
41	OJY48151.1	87	MBV9815528.1	133	WP_169259343.1
42	WP_062397209.1	88	MYH41266.1	134	WP_201494572.1
43	WP_169186431.1	89	MPZ97997.1	135	MBN9621549.1
44	WP_133828190.1	90	MBT5774752.1	136	OZG26106.1
45	MBS0560157.1	91	XP_014714961.1	137	WP_016501746.1
46	PCJ59575.1	92	TAK78428.1		

or
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[0010] Embodiment 7. The method of Embodiment 4, wherein 2,4-dihydroxybutanal is produced by contacting 2,4-dihydroxybutyryl-CoA with an acyl-CoA reductase.

[0011] Embodiment 8. The method of Embodiment 7, wherein the acyl-CoA reductase is selected from:

ACR#	GenBank Accession Number	ACR#	GenBank Accession Number
1	ABX41556.1	26	WP_094899923.1
2	WP_185879480.1	27	WP_152887945.1
3	WP_051457024.1	28	HAR84250.1
4	WP_088269124.1	29	MBS5083929.1
5	WP_087641473.1	30	WP_027296492.1
6	WP_051541705.1	31	WP_104149274.1
7	HHY51863.1	32	WP_135035016.1
8	WP_119112248.1	33	EJO19495.1
9	WP_114642697.1	34	WP_051245790.1
10	WP_202656015.1	35	WP_125552613.1

11	WP_115130814.1	36	HCL03003.1
12	WP_052127368.1	37	WP_126792036.1
13	MBN6206692.1	38	NQJ18683.1
14	WP_051217603.1	39	WP_191507870.1
15	WP_129009770.1	40	MBR5981728.1
16	MTK10086.1	41	WP_215633330.1
17	WP_106009325.1	42	MBP3327663.1
18	HBB29922.1	43	WP_152889336.1
19	WP_216437565.1	44	WP_052356672.1
20	WP_090039956.1	LmACR	Q8Y7U1
21	WP_083963349.1	PtACDH	A0A178TYF6
22	WP_012101452.1	StEutE	P41793
23	WP_122646076.1	RpPduP	Q21A49
24	WP_070791575.1	BmACDH	I3E349
25	WP_010715224.1	CbAld	Q716S8

or
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[0012] Embodiment 9. The method of Embodiment 4, wherein 1,2,4-butanetriol is produced by contacting 2,4-dihydroxybutanal with an alcohol dehydrogenase.

[0013] Embodiment 10. The method of Embodiment 9, wherein the alcohol dehydrogenase is selected from:

Gene	GenBank Accession Number
AdhE	P0A9Q7
AhdE2	Q9ANR5
ALD5	P40047
YqhD	Q46856
YahK	P75691
GLD1	Q0GYU5

or
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[0014] Embodiment 11. The method of Embodiment 4, wherein 4-hydroxybutanal is produced by contacting 1,2,4-butanetriol with a diol dehydratase.

[0015] Embodiment 12. The method of Embodiment 11, wherein the diol dehydratase is selected from:

DDR#	GenBank Accession Number	DDR#	GenBank Accession Number
1	Q59476, O08505, Q59475	16	NP_418386
2	Q8GEZ8, Q8GEZ7	17	ZP_03717880.1
3	P45514, A8CH91, A8CH95	18	YP_356813.1
4	Q59470, Q59471, Q59472	19	WP_011471631.1

5	A0A828U7A4, A0A828U8K0	20	WP_011388658.1
6	ACI39932	21	ABC25539
7	ZP_03292457.1	22	ZP_02040253.1
8	ZP_03706520.1	23	ZP_01962384.1
9	WP_011721311.1	24	ZP_02701301.1
10	WP_012199200.1	25	NP_462994
11	ABW38375	26	WP_011787819.1
12	WP_011099190.1	27	NP_709751
13	WP_015943317.1	28	ZP_00789518.1
14	ZP_03467192.1	29	ZP_01867938.1
15	ZP_02692139.1		

or
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[0016] Embodiment 13. The method of Embodiment 4, wherein 1,4-butanediol is produced by contacting 4-hydroxybutanal with an alcohol dehydrogenase.

[0017] Embodiment 14. The method of Embodiment 13, wherein the alcohol dehydrogenase is selected from:

Gene	GenBank Accession Number
AdhE	P0A9Q7
AhdE2	Q9ANR5
ALD5	P40047
YqhD	Q46856
YahK	P75691
GLD1	Q0GYU5

or
.

[0018] In another aspect, provided herein is a method for producing 1,4-butanediol via condensation of glycolaldehyde and C1-derived formyl-CoA.

[0019] Embodiment 15. A method for producing glycerol from glycolaldehyde comprising condensation of glycolaldehyde with formyl-CoA, comprising:

- (a) forming glyceryl-CoA from glycolaldehyde;
- (b) forming glyceraldehyde from glyceryl-CoA; and
- (c) forming glycerol from glyceraldehyde.

[0020] Embodiment 16. The method of embodiment 15, wherein forming glyceryl-CoA comprises contacting glycolaldehyde and formyl-CoA with a TPP-dependent enzyme selected from 2-hydroxyacyl-CoA synthase, 2-hydroxyacyl-CoA lyase, oxalyl-CoA decarboxylase or benzaldehyde lyase.

[0021] Embodiment 17. The method of embodiment 16, wherein the TPP-dependent enzyme is 2-hydroxyacyl-CoA synthase or 2-hydroxyacyl-CoA lyase.

[0022] Embodiment 18. The method of embodiment 17, wherein the TPP-dependent enzyme is selected from:

HACS#	GenBank Accession Number	HACS#	GenBank Accession Number	HACS#	GenBank Accession Number
1	XP_012756082.1	47	MXY78649.1	93	TAJ19927.1
2	TMK01573.1	48	MBA01399.1	94	PKN81274.1
3	PYM26381.1	49	MXX31676.1	95	RLT34960.1
4	EEG70177.1	50	MXV80929.1	96	MBT5775398.1
5	MBH80817.1	51	MBI4083577.1	97	TMD99851.1
6	WP_030891887.1	52	MBK6319978.1	98	MSQ12864.1
7	AGK93615.1	53	MBI5948182.1	99	MBL0714078.1
8	MAX57815.1	54	PFG74273.1	100	WP_114297888.1
9	WP_068916287.1	55	WP_158065972.1	101	MAK25262.1
10	WP_062165271.1	56	MBN9492325.1	102	WP_068138361.1
11	MBB43458.1	57	MBK6663287.1	103	RMG94145.1
12	PCJ72347.1	58	MBI2766664.1	104	MBA4180234.1
13	TMQ19149.1	59	HEM18354.1	105	MBM3723043.1
14	MAX11513.1	60	GBD22648.1	106	ABF11225.1
15	HAK63664.1	61	MBF6599205.1	107	TAL98798.1
16	MBG92919.1	62	MXW00101.1	108	NNN20496.1
17	PZC46201.1	63	MYA07641.1	109	MBP1761901.1
18	MBB84818.1	64	REJ76484.1	110	PPQ43247.1
19	OGA51379.1	65	HDY15625.1	111	MSQ25793.1
20	PWB41796.1	66	MBW2231087.1	112	TMK28344.1
21	MAE93843.1	67	NRA08835.1	113	HIB12002.1
22	OGP60024.1	68	NQZ98823.1	114	WP_179589464.1
23	OWB57166.1	69	MBI3918747.1	115	MXY42918.1
24	KXN72624.1	70	MBI2761137.1	116	WP_184156128.1
25	PVU86112.1	71	MBE0608783.1	117	HET53513.1
26	ORZ16580.1	72	MYA54281.1	118	TMK22624.1
27	XP_005644825.1	73	NRA01576.1	119	MXX66290.1
28	KZV27770.1	74	MBW2623123.1	120	GIS94895.1
29	EJY87672.1	75	MBI5615765.1	121	MBN1557905.1
30	HIG47824.1	76	MSR14309.1	122	MSV30368.1
31	TMD03111.1	77	XP_004342722.2	123	MBN2179295.1

32	MBJ56818.1	78	MSP42197.1	124	TDI90456.1	
33	WP_095860310.1	79	TDI61101.1	125	OGN76415.1	
34	MBL8483477.1	80	MBO0741576.1	126	WP_102074055.1	
35	WP_058697592.1	81	MBO0736096.1	127	PZC47999.1	
36	WP_130292058.1	82	MBV9828771.1	128	HHH88785.1	
37	WP_207956071.1	83	MAW55136.1	129	OLB93949.1	
38	WP_132429652.1	84	MBV38827.1	130	PKB76696.1	
39	WP_060575023.1	85	TMJ68231.1	131	HED24197.1	
40	WP_068796145.1	86	TMJ64557.1	132	WP_066960443.1	
41	OJY48151.1	87	MBV9815528.1	133	WP_169259343.1	
42	WP_062397209.1	88	MYH41266.1	134	WP_201494572.1	
43	WP_169186431.1	89	MPZ97997.1	135	MBN9621549.1	
44	WP_133828190.1	90	MBT5774752.1	136	OZG26106.1	or
45	MBS0560157.1	91	XP_014714961.1	137	WP_016501746.1	.
46	PCJ59575.1	92	TAK78428.1			

[0023] Embodiment 19. The method of any one of Embodiments 16-18, further comprising forming formyl-CoA from one-carbon (C1) compound.

[0024] Embodiment 20. The method of Embodiment 19, wherein forming formyl-CoA comprises contacting the one-carbon (C1) compound selected from formaldehyde or formate with an enzyme.

[0025] Embodiment 21. The method of Embodiment 20, wherein the C1 compound is formaldehyde, and the enzyme is an acyl-CoA reductase.

[0026] Embodiment 22. The method of Embodiment 21, wherein the acyl-CoA reductase is selected from:

ACR#	GenBank Accession Number	ACR#	GenBank Accession Number
1	ABX41556.1	26	WP_094899923.1
2	WP_185879480.1	27	WP_152887945.1
3	WP_051457024.1	28	HAR84250.1
4	WP_088269124.1	29	MBS5083929.1
5	WP_087641473.1	30	WP_027296492.1
6	WP_051541705.1	31	WP_104149274.1
7	HHY51863.1	32	WP_135035016.1
8	WP_119112248.1	33	EJO19495.1
9	WP_114642697.1	34	WP_051245790.1
10	WP_202656015.1	35	WP_125552613.1
11	WP_115130814.1	36	HCL03003.1

12	WP_052127368.1	37	WP_126792036.1	
13	MBN6206692.1	38	NQJ18683.1	
14	WP_051217603.1	39	WP_191507870.1	
15	WP_129009770.1	40	MBR5981728.1	
16	MTK10086.1	41	WP_215633330.1	
17	WP_106009325.1	42	MBP3327663.1	
18	HBB29922.1	43	WP_152889336.1	
19	WP_216437565.1	44	WP_052356672.1	
20	WP_090039956.1	LmACR	Q8Y7U1	
21	WP_083963349.1	PtACDH	A0A178TYF6	
22	WP_012101452.1	StEutE	P41793	
23	WP_122646076.1	RpPduP	Q21A49	
24	WP_070791575.1	BmACDH	I3E349	or
25	WP_010715224.1	CbAld	Q716S8	.

[0027] Embodiment 23. The method of Embodiment 20, further comprising generating formaldehyde from methanol by contacting methanol with a methanol dehydrogenase or methanol oxidase.

[0028] Embodiment 24. The method of Embodiment 23, wherein the methanol dehydrogenase or methanol oxidase is selected from:

Gene	GenBank Accession Number	
BmMDH2	I3E2P9	
CnMDH2	F8GNE5	
BsMDH	P42327	
MeMxaF-MxaI	P16027, P14775	
CbAOD1	Q00922	
PaMOX	P04841	
PpAOX1	P04842	
PpAOX2	F2R038	
PmMOD1	Q9UVU2	or
PmMOD2	Q9UVU1	.

[0029] Embodiment 25. The method of Embodiment 23 or 24, wherein further comprising generating methanol from methane by contacting methane with a methane monooxygenase.

[0030] Embodiment 26. The method of Embodiment 25, wherein the methane monooxygenases is selected from:

Gene	GenBank Accession Number
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mmoXYBZCD	P18797	or
pmoA1A2B1B2	G1UBD1, Q607G3	

[0031] Embodiment 27. The method of Embodiment 20, wherein the C1 compound is formate and the enzyme is selected from:

- (i) an acyl-CoA transferase catalyzing the conversion of formate to formyl-CoA;
- (ii) an acyl-CoA synthase catalyzing the conversion of formate to formyl-CoA; or
- (iii) a formate kinase catalyzing the conversion of formate to formyl-phosphate and a phosphate formyltransferase catalyzing the conversion of formyl-phosphate to formyl-CoA.

[0032] Embodiment 28. The method of Embodiment 27, wherein the acyl-CoA transferase is selected from:

ACT#	GenBank Accession Number	ACT#	GenBank Accession Number
1	NBW24427.1	33	MBW7889249.1
2	HBE84973.1	34	MBK7676927.1
3	WP_132821656.1	35	MBV8105822.1
4	WP_220287672.1	36	MBP7764230.1
5	WP_121965416.1	37	MSO92582.1
6	XP_014530103.1	38	MBE9574050.1
7	2OAS_1	39	MBX9950024.1
8	WP_084235291.1	40	AEK61848.1
9	MBS6366228.1	41	MBL8096225.1
10	MBR5999861.1	42	OPZ27283.1
11	3D3U_1	43	RYD04206.1
12	WP_073092544.1	44	MBQ9059638.1
13	MBR0090292.1	45	2G39_1
14	MBU4349155.1	46	2NVV_1
15	NLU48536.1	47	MBQ9530926.1
16	3EH7_1	48	4EU3_1
17	MBS0639490.1	49	KAF4531260.1
18	WP_194298948.1	50	2HJ0_1
19	3UBM_A	51	SJZ60628.1
20	WP_203555095.1	52	ALP92681.1
21	MBC7087538.1	53	GFX41336.1
22	WP_191390389.1	54	5VIT_1
23	MBR0127170.1	55	SDR52074.1
24	WP_206582952.1	56	MBF0160244.1

25	MBK5252043.1	57	MBF0445994.1	or .
26	MBR2778738.1	58	HGX18505.1	
27	MBF8291010.1	59	MBK9387745.1	
28	RLI25587.1	60	MBF0310027.1	
29	HHW62298.1	61	RKY20198.1	
30	NWF83872.1	62	NYH33089.1	
31	NUN70050.1	63	Q9RM86	
32	MBU2447274.1	64	O06644	

[0033] Embodiment 29. The method of Embodiment 27, wherein the acyl-CoA synthase is selected from:

Gene	GenBank Accession Number	or .
EcACS	P27550	
BsACS	P39062	
StACSstab	Q8ZKF6	
MhACS	F2NQX2	
ArACS	A0A0Q7JEV7	

[0034] Embodiment 30. The method of Embodiment 27, wherein the formate kinase and the phosphate formyltransferase pair is selected from:

JGIK#	GenBank Accession Number		JGIK#	GenBank Accession Number	
	ACK	PTA		ACK	PTA
1	AKJ38693.1	AKJ38694.1	21	MBU2064158.1	MBU2064159.1
2	BCV24779.1	BCV24778.1	22	MBU2501086.1	MBU2501087.1
3	EDM85332.1	EDM85331.1	23	NLA96479.1	NLA96478.1
4	GFI65571.1	GFI65570.1	24	NLI62094.1	NLI62095.1
5	HBG22385.1	HBG22386.1	25	NLM93282.1	NLM93283.1
6	HFV10353.1	HFV10352.1	26	NMA59030.1	NMA59029.1
7	HGG13858.1	HGG13857.1	27	OGI05344.1	OGI05345.1
8	HIX51076.1	HIX51075.1	28	OHB58473.1	OHB58472.1
9	KXK65140.1	KXK65141.1	29	PWM39673.1	PWM39672.1
10	KXL51791.1	KXL51790.1	30	TKJ47541.1	TKJ47542.1
11	MBE5816841.1	MBE5816840.1	31	WP_022744670.1	WP_022744669.1
12	MBE6451999.1	MBE6451998.1	32	WP_023275423.1	WP_023275424.1
13	MBF0205569.1	MBF0205570.1	33	WP_076546120.1	WP_076546119.1

14	MBI4335478.1	MBI4335477.1	34	WP_078810629.1	WP_078810628.1
15	MBN1633386.1	MBN1633385.1	35	WP_099343330.1	WP_099343331.1
16	MBR2784446.1	MBR2784445.1	36	WP_106012460.1	WP_106012461.1
17	MBR3082232.1	MBR3082233.1	37	AAA72042.1	AAA72041.1
18	MBS5449595.1	MBS5449596.1	38	BAG33697.1	BAG33698.1
19	MBS6942200.1	MBS6942201.1	39	A0A0J8DB00	A0A0J8D6J2
20	MBU0667180.1	MBU0667181.1	40	P0A6A3	P0A9M8

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[0035] Embodiment 31. The method of any one of Embodiments 15-30, wherein forming glyceraldehyde comprising contacting the glyceryl-CoA with an acyl-CoA reductase catalyzing the conversion of glyceryl-CoA to glyceraldehyde.

[0036] Embodiment 32. The method of Embodiment 31, wherein the acyl-CoA reductase is selected from:

ACR#	GenBank Accession Number	ACR#	GenBank Accession Number
1	ABX41556.1	26	WP_094899923.1
2	WP_185879480.1	27	WP_152887945.1
3	WP_051457024.1	28	HAR84250.1
4	WP_088269124.1	29	MBS5083929.1
5	WP_087641473.1	30	WP_027296492.1
6	WP_051541705.1	31	WP_104149274.1
7	HHY51863.1	32	WP_135035016.1
8	WP_119112248.1	33	EJO19495.1
9	WP_114642697.1	34	WP_051245790.1
10	WP_202656015.1	35	WP_125552613.1
11	WP_115130814.1	36	HCL03003.1
12	WP_052127368.1	37	WP_126792036.1
13	MBN6206692.1	38	NQJ18683.1
14	WP_051217603.1	39	WP_191507870.1
15	WP_129009770.1	40	MBR5981728.1
16	MTK10086.1	41	WP_215633330.1
17	WP_106009325.1	42	MBP3327663.1
18	HBB29922.1	43	WP_152889336.1
19	WP_216437565.1	44	WP_052356672.1
20	WP_090039956.1	LmACR	Q8Y7U1

21	WP_083963349.1	PtACDH	A0A178TYF6	or .
22	WP_012101452.1	StEutE	P41793	
23	WP_122646076.1	RpPduP	Q21A49	
24	WP_070791575.1	BmACDH	I3E349	
25	WP_010715224.1	CbAld	Q716S8	

[0037] Embodiment 33. The method of any one of Embodiments 15-32, wherein forming glycerol comprises contacting the glyceraldehyde with an alcohol dehydrogenase.

[0038] Embodiment 34. The method of Embodiment 33, wherein the alcohol dehydrogenase is selected from:

Gene	GenBank Accession Number	or .
AdhE	P0A9Q7	
AhdE2	Q9ANR5	
ALD5	P40047	
YqhD	Q46856	
YahK	P75691	
GLD1	Q0GYU5	.

[0039] Embodiment 35. The method of any one of Embodiments 15-34, further comprising forming glycolaldehyde via oxidation of ethylene glycol.

[0040] Embodiment 36. The method of Embodiment 35, wherein forming glycolaldehyde comprises contacting the ethylene glycol with an alcohol dehydrogenase selected from:

Gene	GenBank Accession Number	or .
EcFucO	P0A9S1	
AdhE	P0A9Q7	
AhdE2	Q9ANR5	
ALD5	P40047	
YqhD	Q46856	
YahK	P75691	
GLD1	Q0GYU5	.

[0041] Also provided herein is a method for producing 1,4-butanediol via condensation of glycolaldehyde and C1-derived formyl-CoA, with the glycolaldehyde being itself produced via condensation of formaldehyde and C1-derived formyl-CoA; and a method for generation of formyl-CoA from C1 compounds such as methane, methanol, formaldehyde or formate.

[0042] Embodiment 37. A method of producing glycolaldehyde from formaldehyde, comprising forming glycolyl-CoA from formyl-CoA and forming glycolaldehyde from glycolyl-CoA.

[0043] Embodiment 38. The method of Embodiment 37, wherein forming glycolyl-CoA comprises contacting formaldehyde and formyl-CoA (generated by the method described in Embodiments 20-30) with a TPP-dependent enzyme selected from 2-hydroxyacyl-CoA synthase, 2-hydroxyacyl-CoA lyase, oxalyl-CoA decarboxylase or benzaldehyde lyase.

[0044] Embodiment 39. The method of Embodiment 38, wherein the TPP-dependent enzyme is 2-hydroxyacyl-CoA synthase or 2-hydroxyacyl-CoA lyase.

[0045] Embodiment 40. The method of Embodiment 38, wherein the TPP-dependent enzyme is selected from:

HACS#	GenBank Accession Number	HACS#	GenBank Accession Number	HACS#	GenBank Accession Number
1	XP_012756082.1	47	MXY78649.1	93	TAJ19927.1
2	TMK01573.1	48	MBA01399.1	94	PKN81274.1
3	PYM26381.1	49	MXX31676.1	95	RLT34960.1
4	EEG70177.1	50	MXV80929.1	96	MBT5775398.1
5	MBH80817.1	51	MBI4083577.1	97	TMD99851.1
6	WP_030891887.1	52	MBK6319978.1	98	MSQ12864.1
7	AGK93615.1	53	MBI5948182.1	99	MBL0714078.1
8	MAX57815.1	54	PFG74273.1	100	WP_114297888.1
9	WP_068916287.1	55	WP_158065972.1	101	MAK25262.1
10	WP_062165271.1	56	MBN9492325.1	102	WP_068138361.1
11	MBB43458.1	57	MBK6663287.1	103	RMG94145.1
12	PCJ72347.1	58	MBI2766664.1	104	MBA4180234.1
13	TMQ19149.1	59	HEM18354.1	105	MBM3723043.1
14	MAX11513.1	60	GBD22648.1	106	ABF11225.1
15	HAK63664.1	61	MBF6599205.1	107	TAL98798.1
16	MBG92919.1	62	MXW00101.1	108	NNN20496.1
17	PZC46201.1	63	MYA07641.1	109	MBP1761901.1
18	MBB84818.1	64	REJ76484.1	110	PPQ43247.1
19	OGA51379.1	65	HDY15625.1	111	MSQ25793.1
20	PWB41796.1	66	MBW2231087.1	112	TMK28344.1
21	MAE93843.1	67	NRA08835.1	113	HIB12002.1
22	OGP60024.1	68	NQZ98823.1	114	WP_179589464.1
23	OWB57166.1	69	MBI3918747.1	115	MXY42918.1

24	KXN72624.1	70	MBI2761137.1	116	WP_184156128.1
25	PVU86112.1	71	MBE0608783.1	117	HET53513.1
26	ORZ16580.1	72	MYA54281.1	118	TMK22624.1
27	XP_005644825.1	73	NRA01576.1	119	MXX66290.1
28	KZV27770.1	74	MBW2623123.1	120	GIS94895.1
29	EJY87672.1	75	MBI5615765.1	121	MBN1557905.1
30	HIG47824.1	76	MSR14309.1	122	MSV30368.1
31	TMD03111.1	77	XP_004342722.2	123	MBN2179295.1
32	MBJ56818.1	78	MSP42197.1	124	TDI90456.1
33	WP_095860310.1	79	TDI61101.1	125	OGN76415.1
34	MBL8483477.1	80	MBO0741576.1	126	WP_102074055.1
35	WP_058697592.1	81	MBO0736096.1	127	PZC47999.1
36	WP_130292058.1	82	MBV9828771.1	128	HHH88785.1
37	WP_207956071.1	83	MAW55136.1	129	OLB93949.1
38	WP_132429652.1	84	MBV38827.1	130	PKB76696.1
39	WP_060575023.1	85	TMJ68231.1	131	HED24197.1
40	WP_068796145.1	86	TMJ64557.1	132	WP_066960443.1
41	OJY48151.1	87	MBV9815528.1	133	WP_169259343.1
42	WP_062397209.1	88	MYH41266.1	134	WP_201494572.1
43	WP_169186431.1	89	MPZ97997.1	135	MBN9621549.1
44	WP_133828190.1	90	MBT5774752.1	136	OZG26106.1
45	MBS0560157.1	91	XP_014714961.1	137	WP_016501746.1
46	PCJ59575.1	92	TAK78428.1		

or
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[0046] Embodiment 41. The method of any one of Embodiments 37-40, wherein forming glycolaldehyde comprises contacting the glycolyl-CoA with an acyl-CoA reductase catalyzing the conversion of glycolyl-CoA to glycolaldehyde.

[0047] Embodiment 42. The method of Embodiment 41, wherein the acyl-CoA reductase is selected from:

ACR#	GenBank Accession Number	ACR#	GenBank Accession Number
1	ABX41556.1	26	WP_094899923.1
2	WP_185879480.1	27	WP_152887945.1
3	WP_051457024.1	28	HAR84250.1
4	WP_088269124.1	29	MBS5083929.1
5	WP_087641473.1	30	WP_027296492.1
6	WP_051541705.1	31	WP_104149274.1
7	HHY51863.1	32	WP_135035016.1
8	WP_119112248.1	33	EJO19495.1

9	WP_114642697.1	34	WP_051245790.1
10	WP_202656015.1	35	WP_125552613.1
11	WP_115130814.1	36	HCL03003.1
12	WP_052127368.1	37	WP_126792036.1
13	MBN6206692.1	38	NQJ18683.1
14	WP_051217603.1	39	WP_191507870.1
15	WP_129009770.1	40	MBR5981728.1
16	MTK10086.1	41	WP_215633330.1
17	WP_106009325.1	42	MBP3327663.1
18	HBB29922.1	43	WP_152889336.1
19	WP_216437565.1	44	WP_052356672.1
20	WP_090039956.1	LmACR	Q8Y7U1
21	WP_083963349.1	PtACDH	A0A178TYF6
22	WP_012101452.1	StEutE	P41793
23	WP_122646076.1	RpPduP	Q21A49
24	WP_070791575.1	BmACDH	I3E349
25	WP_010715224.1	CbAld	Q716S8

or
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[0048] Embodiment 43. A method for producing 1,4-butanediol from C1 compounds such as methane or methanol, comprising forming formaldehyde (for example, by the method described in Embodiments 23-26), forming formyl-CoA (for example, by the method described in Embodiments 20-30), forming glycolaldehyde from formaldehyde (for example, by the method described in Embodiments 37-42), forming glycerol from glycolaldehyde (for example, by the method described in Embodiments 15-36), and the conversion of glycerol to 1,4-butanediol (for example, by the method described in Embodiments 1-14).

[0049] Embodiment 44. The method of any one of Embodiments 1 to 43, wherein the enzyme used in the method is isolated from a microorganism.

[0050] Embodiment 45. The method of any one of Embodiments 1 to 43, wherein the enzyme used in the method is contained in a microorganism.

[0051] Embodiment 46. A genetically modified microorganism providing 1,4-butanediol from glycerol by the method of any one of Embodiments 2 to 14.

[0052] Embodiment 47. A genetically modified microorganism further providing glycerol by the method of any one for Embodiment 15 to 36.

[0053] Embodiment 48. A genetically modified microorganism further providing glycerol from C1 compounds by the method of any one for Embodiment 31 to 36.

[0054] Embodiment 49. The microorganism of any one of Embodiments 46 to 48, wherein the microorganism is selected from the group consisting of bacteria, yeast and fungi.

[0055] Embodiment 50. The microorganism of any one of Embodiments 46 to 49, wherein the microorganism is bacteria, yeast or fungi, including but not limited to *Escherichia sp.*, *Bacillus sp.*, *Pseudomonas sp.*, *Corynebacterium sp.*, *Zymonas sp.*, *Clostridium sp.*, *Streptococcus sp.*, *Rhodococcus sp.*, *Geobacillus sp.*, *Saccharomyces sp.*, *Pichia sp.*, *Yarrowia sp.*, *Methylobacterium sp.*, *Candida sp.*, *Kluyveromyces sp.*, *Aspergillus sp.*, *Penicillium sp.*, *Rhizopus sp.* and *Trichoderma sp.*

[0056] The following description of examples provides additional details, any one of which can be subject to patenting in combination with any other. The specification in its entirety is to be treated as providing a variety of details that can be used interchangeably with other details.

[0057] The present disclosure is illustrated by the following non-limiting examples.

[0058] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only, and are not restrictive of the invention. Further, the accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate embodiments of the invention and together with the description, serve to explain principles of the invention.

BRIEF DESCRIPTION OF DRAWINGS

[0059] The drawings referenced herein form a part of the specification. Features shown in the drawing illustrate only some embodiments of the application, and not of all embodiments of the application, unless the detailed description explicitly indicates otherwise, and readers of the specification should not make implications to the contrary.

[0060] Figure 1 is a scheme illustrating the interconversion of C1 compounds. A) Production of formyl-CoA from C1 compounds; B) GSH- and MSH-dependent oxidation of formaldehyde to formate; C) H₄F and H₄MPT-dependent oxidation of formaldehyde to formate.

[0061] MMO: methane monooxygenase; MDH: methanol dehydrogenase; AOD: alcohol oxidase; FOK: formate kinase; PTA: phosphate formyltransferase; ACS: acyl-CoA synthase; ACT: acyl-CoA transferase; ACR: acyl-CoA reductase; HACS: 2-hydroxyacyl-CoA synthase; TE: thioesterase; FLD: formaldehyde dehydrogenase; FGH: formyl-GS hydrolase; FHC: formyltransferase/ hydrolase; MTDA: methylene-tetrahydromethanopterin dehydrogenases; FCH: methenyl-H₄F cyclohydrolase; FTFL: formate-H₄F ligase; FAE: formaldehyde activating; MCH: methenyl-H₄MPT cyclohydrolase enzyme; MCH: methenyl-H₄MPT cyclohydrolase.

[0062] Figure 2 is a scheme illustrating the generation of formyl-CoA from formate and the generation of glycolate from the condensation of formaldehyde and formyl-CoA. A) shows the reaction driven by ACS and HACS; B) shows the reaction driven by a set of FOK-PTA and HACS.

[0063] ACS: acyl-CoA synthase (BsACS: *Bacillus subtilis*, EcACS: *Escherichia coli*, MhACS: *Marinithermus hydrothermalis*, StACSstab: *Salmonella typhimurium*), ApbHCAS: 2-hydroxyacyl-CoA synthase derived from Alphaproteobacteria bacterium, FOK-PTA: formate kinase-phosphate formyltransferase derived from Coriobacteriia bacterium and DtbHACS: 2-hydroxyacyl-CoA synthase derived from Deltaproteobacteria bacterium.

[0064] Figure 3 is a scheme illustrating the production of 1,4-butanediol from glycerol coupled to generation of formyl-CoA and reducing equivalents from C1 compounds.

[0065] MMO: methane monooxygenase; MDH: methanol dehydrogenase; AOD: alcohol oxidase; FOK: formate kinase; PTA: phosphate formyltransferase; ACS: acyl-CoA synthase; ACT: acyl-CoA transferase; ACR: acyl-CoA reductase; HACS: 2-hydroxyacyl-CoA synthase; ADH: alcohol dehydrogenase; DDR: diol dehydratase; GDHt: glycerol dehydratase.

[0066] Figure 4 is a scheme illustrating the pathway design for 1,4-butanediol via C1+BioTM platform. A) A whole cell bioconversion of glycerol to 3-hydroxypropionaldehyde by GDHt, measured by colorimetric assay; B) HPLC chromatogram of cell-free bioconversion samples through the condensation of 3-hydroxypropionaldehyde and formyl-CoA catalyzed by ApbHACS.

[0067] KpGDHt: glycerol dehydratase derived from *Klebsiella pneumoniae*, GdrAB: activator protein, ApbHACS: 2-hydroxyacyl-CoA synthase derived from Alphaproteobacteria bacterium, ACT: acyl-CoA transferase, and TE: thioesterase. The HPLC system is operated by using Coregel 107H column (250 mm x4.6 mm, 5 µm) with flow rate of 0.5 mL/min using 10 mM of sulfuric acid as a mobile phase. Detection was performed at 214 nm using photodiode array detector and retention times of 2,4-dihydroxybutyric acid is around 17.9 min.

[0068] Figure 5 is a scheme illustrating the production of 1,4-butanediol from ethylene glycol coupled to generation of formyl-CoA and reducing equivalents from C1 compounds.

[0069] MMO: methane monooxygenase; MDH: methanol dehydrogenase; AOD: alcohol oxidase; FOK: formate kinase; PTA: phosphate formyltransferase; ACS: acyl-CoA synthase; ACT: acyl-CoA transferase; ACR: acyl-CoA reductase; HACS: 2-hydroxyacyl-CoA synthase; ADH: alcohol dehydrogenase; DDR: diol dehydratase; GDHt: glycerol dehydratase; FucO: lactaldehyde reductase.

[0070] Figure 6 is a scheme illustrating the production of 1,4-butanediol from C1 compounds coupled to generation of formyl-CoA and reducing equivalents from C1 compounds.

[0071] MMO: methane monooxygenase; MDH: methanol dehydrogenase; AOD: alcohol oxidase; FOK: formate kinase; PTA: phosphate formyltransferase; ACS: acyl-CoA synthase; ACT: acyl-CoA transferase; ACR: acyl-CoA reductase; HACS: 2-hydroxyacyl-CoA synthase; ADH: alcohol dehydrogenase; DDR: diol dehydratase; GDHt: glycerol dehydratase.

[0072] **DETAILED DESCRIPTION OF THE INVENTION**

[0073] The following detailed description of exemplary embodiments of the application refers to the accompanying drawings that form a part of the description. The drawings illustrate specific exemplary embodiments in which the application may be practiced. The detailed description, including the drawings, describes these embodiments in sufficient detail to enable those skilled in the art to practice the application. Those skilled in the art may further utilize other embodiments of the application, and make logical, mechanical, and other changes without departing from the spirit or scope of the application. Readers of the following detailed description should, therefore, not interpret the description in a limiting sense, and only the appended claims define the scope of the embodiment of the application.

[0074] In this application, the use of the singular includes the plural unless specifically stated otherwise. In this application, the use of “or” means “and/or” unless stated otherwise. Furthermore, the use of the term “including” as well as other forms such as “includes” and “included” is not limiting. Additionally, the section headings used herein are for organizational purposes only, and are not to be construed as limiting the subject matter described.

[0075] **EXAMPLE 1: INTERCONVERSION OF ONE-CARBON COMPOUNDS TO GENERATE FORMYL-COA**

[0076] Formyl-CoA can be provided through the interconversion of C1 compounds including methane, methanol, formaldehyde, and formate (**Figure 1** below) with suitable enzymes (e.g., enzymes set forth in **Table 4** below). Methane is readily available C1 source from natural gas, landfills, and agriculture. Biological oxidation of methane to methanol is catalyzed by methane monooxygenases. Functional expression of soluble methane monooxygenase (sMMO) from *Methylococcus capsulatus* is demonstrated in *E. coli* as a host (bioRxiv 2021. 08.05.455234). Subsequent oxidation of methanol to formaldehyde can be catalyzed by NAD⁺-dependent methanol dehydrogenases (MDH), MDH from *Bacillus methanolicus* MGA3 (BmMDH), *Bacillus stearothermophilus* (BsMDH) (Metab. Eng. 39:49-59, 2017) and *Cupriavidus necator* (Appl. Microbiol. Biotechnol. 100:4969-4983, 2016), PQQ (pyrroloquinoline quinone)-

dependent MDH (MxaFI, or XoxF), or oxygen-dependent alcohol oxidase (AOD). When AOD is used, a catalase (CTA) is needed for de-toxicity of hydrogen peroxide (e.g., enzymes set forth in **Table 4** below).

[0077] Formaldehyde can be directly oxidized to formic acid catalyzed by four cofactor-dependent formaldehyde oxidation pathways, involving glutathione (GSH), tetrahydrofolate (H₄F, THF), tetrahydromethanopterin (H₄MPT), or mycothiol (MSH). Formaldehyde spontaneously reacts with glutathione (GSH) to form S-hydroxymethyl-GS. Hydroxymethyl-GS is then converted to formyl-GS by hydroxymethyl-GS dehydrogenase (FrmA). Formyl-GS is then converted to formate by formyl-GS hydrolase (FrmB). Formaldehyde also spontaneously reacts with mycothiol to form an S-hydroxymethylmycothiol same as GSH-dependent route, and then it was converted to S-formylmycothiol by MD-FalDH, which is further hydrolyzed to formate and MSH via a hydrolase. When H₄MPT was used as the cofactor, formaldehyde condensed with H₄MPT to generate methylene-H₄MPT spontaneously or by formaldehyde activating enzyme (FAE), methylene-H₄MPT is then converted to methenyl-H₄MPT by methylene-tetrahydromethanopterin dehydrogenases MtdA or MtdB. Methenyl-H₄MPT was converted to formyl-H₄MPT by methenyl-H₄MPT cyclohydrolase (MCH), and then converted to formate with the help of formyltransferase/hydrolase FHC. Formaldehyde spontaneously reacts with H₄F to form methylene-H₄F, and then contacting with MtdA it was converted to methenyl-H₄F which is then converted to formyl-H₄F by methenyl-H₄F cyclohydrolase. Formyl-H₄F can be further converted to formate by formate-H₄F ligase FTFH.

[0078] Alternatively, formaldehyde oxidation to formyl-CoA can be catalyzed by various acylating aldehyde dehydrogenase candidates (Nat. Chem. Biol. 15:900-906, 2019). Formyl-CoA can either be fed to the C1 elongation platform or further converted to CO₂ to generate energy. Formyl-CoA hydrolysis to formate can be catalyzed by two different families of enzymes. Acyl-CoA transferases (ACT, e.g., set forth in **Table 5** below) can catalyze reversible CoA transfer from various CoA donor, such as 2,4-dihydroxybutyryl-CoA or acetyl-CoA to formate. A set of phosphotransacylase-formate kinase (PTA-FOK, e.g., set forth in **Table 6** below) pair can also catalyze a reversible phosphorylation of formyl-CoA to formyl-phosphate, followed by dephosphorylation to formate generating 1 ATP. While these two reactions are considered fully reversible, AMP-forming acyl-CoA synthetases are favored toward formate activation to formyl-CoA (ACS, e.g., set forth in **Table 4** below).

[0079] We have demonstrated various pathways for formyl-CoA generation, with glycolate formed through the condensation of formaldehyde and formyl-CoA serving as a representative

indicator. To generate formyl-CoA from formate, a 1 mL of cell-free bioconversion reaction contains 100 mM of potassium phosphate buffer (pH 6.9), 10 mM of MgCl₂, 150 μM TPP, 10 mM of CoA, 50 mM of formate, 10 mM of formaldehyde, 5 μM of HACS and 1 μM of ACS. The reaction mixture was incubated at room temperature for overnight, and the reaction was terminated by adding 2.5 μL of 10 M NaOH then incubated at room temperature for 30 minutes. A 2.5 μL of 10 M HCl was then added to neutralize the pH. The reaction mixture was centrifuged at 12,000 rpm for 2 minutes and analyzed with HPLC. The presence of glycolate from the reaction indicates that formyl-CoA could be generated from formate via the activation of ACS (acyl-CoA synthase) (**Figure 2A** below). In addition to the cell-free bioconversion system, we investigated formyl-CoA generation *in vivo* using *Escherichia coli* MG1655 expressing ACS and HACS. The cells were cultured in TB medium at 37 °C for 2.5 hours with a shaking speed of 200 rpm. Subsequently, 50 μL of 0.1 M IPTG was added, and the culture was further incubated at 30 °C for 24 hours to induce protein expression. The cells were then centrifuged at 5,000 rpm, 4 °C for 5 minutes, and the supernatant was discarded. A 10 mL M9 wash buffer was added to the tube, and the mixture was thoroughly mixed. This washing step was repeated twice. The cells were resuspended in M9 wash buffer to achieve a cell density of OD₆₀₀ = 50; the final cell density in the reaction was OD₆₀₀ = 10. Formaldehyde and formate were initially added to the reaction at final concentrations of 10 mM and 20 mM, respectively. The mixture was incubated at 30 °C for 3.5 hours with a shaking speed of 200 rpm. A 1 mL sample of the reaction mixture was collected for HPLC analysis. Additional formaldehyde and formate, both at final concentrations of 10 mM and 20 mM, were added to the reaction. At 6 hours, formate at a final concentration of 20 mM was reintroduced. The reaction was prolonged to 20 hours and analyzed by HPLC. Glycolate was observed in the reaction, indicating the generation of formyl-CoA from formate *in vivo* by selected ACS (**Figure 2A** below).

[0080] Furthermore, we have tested the formyl-CoA generation from formate via FOK-PTA route. A 1 mL of cell-free bioconversion reaction contains 100 mM of potassium phosphate buffer (pH 6.9), 10 mM of MgCl₂, 150 μM TPP, 10 mM of CoA, 50 mM of formate, 20 mM of formaldehyde, 2 μM of HACS, 2 μM of ACK (FOK) and 2 μM of PTA. The reaction mixture was incubated, terminated, and analyzed in the same manner as mentioned above. The HPLC result showed that 1.45 mM of glycolate was produced from the reaction, confirming the generation of formyl-CoA from formate, catalyzed by a set of FOK-PTA (formyl-CoA kinase and phosphotransacylase) (**Figure 2B** below).

EXAMPLE 2: PRODUCTION OF 1,4-BUTANEDIOL FROM GLYCEROL THROUGH CONDENSATION OF 3-HYDROXYPROPIONALDEHYDE AND FORMYL-COA

[0081] This example demonstrates the implementation of 1,4-butanediol production through C1+Bio™ platform by condensation of aldehydes and formyl-CoA. Feedstocks of varying carbon lengths can be incorporated into the 1,4-butanediol production pathway through different aldehyde intermediates, such as 3-hydroxypropionaldehyde, glycolaldehyde and formaldehyde. When glycerol is utilized as the feedstock for generating 1,4-butanediol, it becomes necessary to enhance the expression of glycerol dehydratase (GDHt) such as KpDhaB123 from *Klebsiella pneumoniae* (GenBank accession: Q59476, O08505, Q59475) (e.g., GDHt set forth in **Table 3** below) to convert glycerol to 3-hydroxypropionaldehyde (**Figure 3** below). 3-hydroxypropionaldehyde is thereafter condensed with formyl-CoA through the activation of HACS that includes BsmHACS (GenBank accession: HAK63664.1), DhCHACS from *Dehalococcoidia bacterium* (GenBank accession: PWB41796.1), CfhHACS from *Chloroflexi bacterium* (GenBank accession: PKN81274.1) and other variants (e.g., HACS set forth in **Table 1** below) to form 2,4-dihydroxybutyryl-CoA for 1,4-butanediol production.

[0082] 2,4-dihydroxybutyryl-CoA is subsequently converted to 2,4-dihydroxybutanal via the activation of acyl-CoA reductase (ACR) (e.g., ACR set forth in **Table 2** below). Alcohol dehydrogenase (ADH) such as enzyme from *Escherichia coli* (EcYqhD, GenBank accession: Q46856) and other ADH variants (e.g., ADH set forth in **Table 3** below) drives the reaction that converts 2,4-dihydroxybutanal to 1,2,4-butanetriol. 1,2,4-butanetriol is then converted to 4-hydroxybutanal by diol dehydratase (DDR) from *Klebsiella oxytoca* (KoPddABC, GenBank accession: Q59470, Q59471, Q59472) and other DDR variants (e.g., DDR set forth in **Table 3** below). The final step is a conversion of 4-hydroxybutanal to 1,4-butanediol by alcohol dehydrogenase (**Figure 2** below; enzymes set forth in **Table 3** below).

[0083] Whole cell bioconversion was carried out to convert glycerol to 3-hydroxypropionaldehyde through the activation of glycerol dehydratase (GDHt). *Escherichia coli* BL21 expressing GDHt derived from *Klebsiella pneumoniae* with the activator protein, GdrA/B was grown in LB medium. The total volume of 1 mL of reaction mixture contains a cell suspension with an OD₆₀₀ of 30, M9 medium, 50 mM of glycerol, 5 mM of KCl, 5 mM of MgCl₂, and 15 μM of Coenzyme B12. The mixture was incubated at 30°C for 5 hours. The reaction mixture was centrifuged at 12,000 rpm for 10 mins and subjected to a colorimetric assay to measure the concentration of 3-hydroxypropionaldehyde. A 100 μL of the supernatant

was mixed with 75 μ L of 0.01 M DL-tryptophan solution (Sigma-Aldrich) in 0.05 M HCl and 300 μ L of 37% v/v HCl. The reaction mixture was incubated at 37°C for 20 mins and measured using a spectrophotometer at an absorbance of 560 nm. 3-hydroxypropionaldehyde from Aikon Chem was used as a standard. The colorimetric assay results demonstrate the conversion of glycerol to 3-hydroxypropionaldehyde, catalyzed by the selected GDHt (**Figure 4A** below).

[0084] Furthermore, we demonstrate the production of 2,4-dihydroxybutyryl-CoA through the condensation of 3-hydroxypropionaldehyde and formyl-CoA using 2,4-dihydroxybutyric acid as a benchmark. A 1 mL of cell-free bioconversion reaction contains 100 mM of potassium phosphate buffer (pH 6.9), 10 mM of $MgCl_2$, 150 μ M TPP, 2 mM of acetyl-CoA, 20 mM of formate, 10 mM of 3-hydroxypropionaldehyde, 2 μ M of HACS and 1 μ M of CaAbfT. The mixture incubated at room temperature for 18 h, and the reaction was terminated by adding 4 μ L of 10 M NaOH. Subsequently, the supernatant was analyzed by HPLC. From the preliminary experiment, we observed the potential formation of 2,4-dihydroxybutyric acid as a sequential product of 2,4-dihydroxybutyryl-CoA, obtained from the condensation of 3-hydroxypropionaldehyde and formyl-CoA, catalyzed by the selected HACS variants (**Figure 4B** below).

EXAMPLE 3: PRODUCTION OF GLYCEROL THROUGH CONDENSATION OF GLYCOLALDEHYDE AND FORMYL-COA

[0085] This example demonstrates the implementation of glycerol production through C1+Bio™ platform by condensation of glycolaldehyde and formyl-CoA (**Figure 5** below). The condensation of glycolaldehyde and formyl-CoA can be catalyzed by HACS (e.g., HACS set forth in **Table 1** below), including but not limited to beach sand metagenome BsmHACS (Genbank accession: HAK63664.1), DhHACS from *Dehalococcoidia bacterium* (Genbank accession: PWB41796.1), CfhHACS from *Chloroflexi bacterium* (Genbank accession: PKN81274.1), to generate glyceryl-CoA.

[0086] Glyceryl-CoA is subsequently converted to glyceraldehyde via the activation of acyl-CoA reductase (ACR) from *Salmonella typhimurium* (StEutE, Genbank accession: P41793) or other ACR variants (e.g., ACR set forth in **Table 2** below). Alcohol dehydrogenases (ADH) such as enzyme from *Escherichia coli* (EcYqhD, Genbank accession: Q46856) drives the reaction that converts glyceraldehyde to glycerol.

[0087] EXAMPLE 4: PRODUCTION OF GLYCOLALDEHYDE FROM ETHYLENE GLYCOL AND ONE-CARBON

[0088] This example demonstrates the glycolaldehyde production from different feed stocks, including but not limited to ethylene glycol (C2) and methanol (C1). When glycolaldehyde is produced, it can be further condensed with formyl-CoA that is generated from C1 compounds to form glyceryl-CoA which will be converted to glycerol, and finally converted to 1,4-butanediol as presented in **Figure 6**.

[0089] Ethylene glycol is firstly converted to glycolaldehyde by *E. coli* FucO or other ADH variants (e.g., ADH set forth in **Table 3**). Glycolaldehyde can be condensed with formyl-CoA to generate glyceryl-CoA that catalyzed by HACS, including CfhHACS from *Chloroflexi bacterium* (GenBank accession: PKN81274.1), RhbHACS from *Rhodocyclaceae* bacterium (GenBank accession: MBL8483477.1), PdsHACS from *Pseudonocardia sediminis* (GenBank accession: WP_130292058.1) or other HACS variants (e.g., HACS set forth in **Table 1** below).

[0090] When methanol or other C1 compounds are used as feedstock, they are firstly converted to formaldehyde and formyl-CoA through the interconversion module. Formaldehyde and formyl-CoA condensation lead to generation of glycolyl-CoA by HACS activation, including CfhHACS from *Chloroflexi bacterium* (GenBank accession: PKN81274.1), RhbHACS from *Rhodocyclaceae* bacterium (GenBank accession: MBL8483477.1), PdsHACS from *Pseudonocardia sediminis* (GenBank accession: WP_130292058.1) or other HACS variants (e.g., HACS set forth in **Table 1** below), and further convert to glycolaldehyde with ACR variants from *Salmonella typhimurium* (StEutE, GenBank accession: P41793) or other ACR variants (e.g., ACR set forth in **Table 2** below).

[0091] EXAMPLE 5: OVERVIEW OF METHODS USED FOR GENETIC MANIPULATION

[0092] The genes for overexpression are either cloned into appropriate vectors or inserted into chromosome with strong synthetic constitutive promoter, such as M1-93. When cloned into vectors, these genes are amplified through PCR using appropriate primers to append homology on each end for recombination into the vector backbone with e.g., Phusion polymerase (Thermo Scientific, Waltham, MA) to serve as the gene insert. Plasmids are linearized by the appropriate restriction enzymes (New England Biolabs, Ipswich, MA, USA) and recombined with the gene inserts using the In-Fusion HD Eco-Dry Cloning system. The mixture is subsequently transformed into Stellar competent cells. Transformants that grow on solid media (LB+Agar) supplemented with the appropriate antibiotic are isolated and screened for the gene insert by PCR. Plasmids from verified transformants are isolated and the sequence of the gene insert is

further confirmed by DNA sequencing. The sequence confirmed plasmids are then introduced to host strain through electroporation.

[0093] When inserted into chromosome, CRISPR is used and genetic sites of *tesB* and *adhE* are suitable loci, although others could be used. The CRISPR method is based on the method developed by Jiang et al. (Jiang, Y., et al. Appl. Environ. Microbiol. 81:2506-2514 (2015)). First, the host strain is transformed with plasmid pCas, the vector for expression of Cas9 and λ -red recombinase. The resulting strain is grown under 30°C with L-arabinose for induction of λ -red recombinase expression, and when OD reaches ~0.6, competent cells are prepared and transformed with pTargetF (AddGene 62226) expressing sgRNA and N20 spacer targeting the locus and template of insertion of target gene. The template is the inserted gene plus M1-93 promoter with ~500 bp sequences homologous with upstream and downstream of the insertion locus, constructed through overlap PCR with usage of Phusion polymerase or synthesized by GenScript (Piscataway, NJ) or GeneArt® (Life Technologies, Carlsbad, CA). The way to switch N20 spacer of pTargetF plasmid is inverse PCR with the modified N20 sequence hanging at the 5' end of primers with usage of Phusion polymerase and followed by self-ligation with usage of T4 DNA ligase and T4 polynucleotide kinase (New England Biolabs, Ipswich, MA, USA). Transformants that grow under 30°C on solid media (LB+Agar) supplemented with spectinomycin and kanamycin (or other suitable antibiotic) are isolated and screened for the chromosomal gene insert by PCR. The sequence of the gene insert, which is amplified from genomic DNA through PCR using Phusion polymerase, is further confirmed by DNA sequencing. The pTargetF can then be cured through IPTG induction, and pCas can be cured through growth under higher temperature like 37-42°C.

[0094] All molecular biology techniques are performed with standard methods (Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989.) or by manufacturer protocol. Strains are stored in glycerol stocks at -80°C. Plates are prepared using LB medium containing 1.5% agar, and appropriate antibiotics are included at the following concentrations: ampicillin (100 µg/mL), kanamycin (50 µg/mL), spectinomycin (50 µg/mL) and chloramphenicol (12.5 µg/mL).

[0095] Expression of selected enzyme variants was achieved using plasmid-based gene expression by cloning the desired gene(s) into pETDuet-1 or pCDFDuet-1 (Novagen, Darmstadt, Germany) digested with appropriate restriction enzymes and by utilizing In-Fusion cloning technology (Clontech Laboratories, Inc., Mountain View, CA). Linear DNA fragments for insertion were created via PCR of the open reading frame of interest (for genes native to *E. coli*) or by gene synthesis of the codon optimized gene. Genes were synthesized by GeneArt

(Life Technologies, Carlsbad, CA). Resulting In-Fusion reaction products were used to transform *E. coli* Stellar cells (Clontech Laboratories, Inc., Mountain View, CA), and clones identified by PCR screening were further confirmed by DNA sequencing. As a host for these vectors, an engineered strain of *E. coli* based on MG1655(DE3), BW25113 or ATCC8739 with knockouts for formaldehyde ($\Delta frmA$), formate ($\Delta fdhF \Delta fdnG \Delta fdoG$) oxidation and aldehyde consumption (including *adhP*, *yjgB*, *yqhD*, *adhE*, etc) can be used, which we expected could compete or interfere with the analysis of our pathway.

[0096] EXAMPLE 6: FERMENTATION CONDITIONS USED FOR IN VIVO PRODUCTION OF 1,4-BUTANEDIOL

[0097] MOPS minimal medium (Neidhardt et al. J. Bacteriol. 119:736-47 (1974)) with 125 mM MOPS and Na₂HPO₄ in place of K₂HPO₄ (2.8 mM), supplemented with 20 g/L glucose, 10 g/L tryptone, 5 g/L yeast extract, 100 μ M FeSO₄, 5 mM (NH₄)₂SO₄, and 30 mM NH₄Cl is used for fermentations. If required, 55 g/L of CaCO₃ is also supplemented as pH buffer. 20 mM lactic acid is supplemented, if it is not synthesized intracellularly and needed for the experiment. 500 mM methanol is also supplemented. Antibiotics (50 μ g/mL carbenicillin, 50 μ g/mL spectinomycin and 50 μ g/mL kanamycin) are included when appropriate. All chemicals are obtained from Fisher Scientific Co. (Pittsburg, PA) and Sigma-Aldrich Co. (St. Louis, MO).

[0098] Fermentations are performed in 25 mL Pyrex Erlenmeyer flasks (narrow mouth/heavy duty rim, Corning Inc., Corning, NY) or 96 deep well plates (2.2 mL, v bottom, USA scientific) filled with appropriate volume of fermentation medium and sealed with foam plugs filling the necks. For anaerobic conditions, 17.5 mL Hungate tubes are filled with fermentation medium and sealed with tubber septa. A single colony of the desired strain is cultivated overnight (14-16 hrs) in LB medium with appropriate antibiotics and used as the inoculum with initial OD₆₀₀ as ~0.05. After inoculation, flasks are incubated in a NBS I24 Benchtop Incubator Shaker (New Brunswick Scientific Co., Inc., Edison, NJ) at 200 rpm and 37°C or 30°C. When optical density (550 nm, OD₅₅₀) reached ~0.3-0.5, appropriate concentration of isopropyl beta-D-1-thiogalactopyranoside (IPTG) (or other suitable inducer) is added for plasmid gene induction. Additional fermentations are conducted in a SixFors multi-fermentation system (Infors HT, Bottmingen, Switzerland) with an air or argon flowrate of 2 N L/hr, independent control of temperature (37 °C), pH (controlled at 7.0 with NaOH and H₂SO₄), and appropriate stirrer speed. Pre-cultures are grown in 25 mL Pyrex Erlenmeyer flasks as described above and incubated for 4 hours post-induction. An appropriate amount of this pre-culture is centrifuged, washed twice with fresh media, and used for inoculation (400 mL initial volume). The fermentations in bioreactor use described fermentation media with 40 g/L glucose and

appropriate IPTG and antibiotics. If required, lactic acid (20 mM) is added at 0, 24, and 48 hours.

[0099] After the fermentation, the supernatant obtained through 5000 g, 5 min centrifuge in an Optima L-80XP Ultracentrifuge (Beckman-Coulter, Schaumburg, IL) of 2 mL culture is prepared for HPLC analysis.

[0100] EXAMPLE 7: HPLC ANALYSIS OF 1,4-BUTANEDIOL

[0101] A 375 μ L of 0.1 M KH_2PO_4 buffer at pH 8.0 and 500 μ L of ethyl acetate containing 4 mM of n-decane were added to 125 μ L of a cell-free reaction mixture saturated with NaCl. The mixture was thoroughly mixed using a vortex and centrifuged at 13,680g for 1 min. The organic phase was dried over anhydrous Na_2SO_4 and then directly used for GC analysis. GC analysis was conducted on a Shimadzu SPL-2014 system equipped with FID-2014 detector and an Agilent DB-5 GC column (30 mm*0.25 mm, 0.25 μ m). Injector temperature was maintained at 250 °C with the flow rate of 1 mL/min. Temperature program was set as 80°C/hold 5 min; 20°C per min to 250 °C, and hold 5 min.

[0102] Table 1. List of 2-hydroxyacyl-CoA synthase (HACS) used for the condensation of 3-hydroxypropionaldehyde and formyl-CoA

HACS#	GenBank Accession Number	HACS#	GenBank Accession Number	HACS#	GenBank Accession Number
1	XP_012756082.1	47	MXV78649.1	93	TAJ19927.1
2	TMK01573.1	48	MBA01399.1	94	PKN81274.1
3	PYM26381.1	49	MXX31676.1	95	RLT34960.1
4	EEG70177.1	50	MXV80929.1	96	MBT5775398.1
5	MBH80817.1	51	MBI4083577.1	97	TMD99851.1
6	WP_030891887.1	52	MBK6319978.1	98	MSQ12864.1
7	AGK93615.1	53	MBI5948182.1	99	MBL0714078.1
8	MAX57815.1	54	PFG74273.1	100	WP_114297888.1
9	WP_068916287.1	55	WP_158065972.1	101	MAK25262.1
10	WP_062165271.1	56	MBN9492325.1	102	WP_068138361.1
11	MBB43458.1	57	MBK6663287.1	103	RMG94145.1
12	PCJ72347.1	58	MBI2766664.1	104	MBA4180234.1
13	TMQ19149.1	59	HEM18354.1	105	MBM3723043.1
14	MAX11513.1	60	GBD22648.1	106	ABF11225.1
15	HAK63664.1	61	MBF6599205.1	107	TAL98798.1
16	MBG92919.1	62	MXW00101.1	108	NNN20496.1
17	PZC46201.1	63	MYA07641.1	109	MBP1761901.1

18	MBB84818.1	64	REJ76484.1	110	PPQ43247.1
19	OGA51379.1	65	HDY15625.1	111	MSQ25793.1
20	PWB41796.1	66	MBW2231087.1	112	TMK28344.1
21	MAE93843.1	67	NRA08835.1	113	HIB12002.1
22	OGP60024.1	68	NQZ98823.1	114	WP_179589464.1
23	OWB57166.1	69	MBI3918747.1	115	MXY42918.1
24	KXN72624.1	70	MBI2761137.1	116	WP_184156128.1
25	PVU86112.1	71	MBE0608783.1	117	HET53513.1
26	ORZ16580.1	72	MYA54281.1	118	TMK22624.1
27	XP_005644825.1	73	NRA01576.1	119	MXX66290.1
28	KZV27770.1	74	MBW2623123.1	120	GIS94895.1
29	EJY87672.1	75	MBI5615765.1	121	MBN1557905.1
30	HIG47824.1	76	MSR14309.1	122	MSV30368.1
31	TMD03111.1	77	XP_004342722.2	123	MBN2179295.1
32	MBJ56818.1	78	MSP42197.1	124	TDI90456.1
33	WP_095860310.1	79	TDI61101.1	125	OGN76415.1
34	MBL8483477.1	80	MBO0741576.1	126	WP_102074055.1
35	WP_058697592.1	81	MBO0736096.1	127	PZC47999.1
36	WP_130292058.1	82	MBV9828771.1	128	HHH88785.1
37	WP_207956071.1	83	MAW55136.1	129	OLB93949.1
38	WP_132429652.1	84	MBV38827.1	130	PKB76696.1
39	WP_060575023.1	85	TMJ68231.1	131	HED24197.1
40	WP_068796145.1	86	TMJ64557.1	132	WP_066960443.1
41	OJY48151.1	87	MBV9815528.1	133	WP_169259343.1
42	WP_062397209.1	88	MYH41266.1	134	WP_201494572.1
43	WP_169186431.1	89	MPZ97997.1	135	MBN9621549.1
44	WP_133828190.1	90	MBT5774752.1	136	OZG26106.1
45	MBS0560157.1	91	XP_014714961.1	137	WP_016501746.1
46	PCJ59575.1	92	TAK78428.1		

[0103] Table 2. List of acyl-CoA reductase (ACR)

ACR#	GenBank Accession Number	ACR#	GenBank Accession Number
1	ABX41556.1	26	WP_094899923.1
2	WP_185879480.1	27	WP_152887945.1
3	WP_051457024.1	28	HAR84250.1
4	WP_088269124.1	29	MBS5083929.1
5	WP_087641473.1	30	WP_027296492.1
6	WP_051541705.1	31	WP_104149274.1

7	HHY51863.1	32	WP_135035016.1
8	WP_119112248.1	33	EJO19495.1
9	WP_114642697.1	34	WP_051245790.1
10	WP_202656015.1	35	WP_125552613.1
11	WP_115130814.1	36	HCL03003.1
12	WP_052127368.1	37	WP_126792036.1
13	MBN6206692.1	38	NQJ18683.1
14	WP_051217603.1	39	WP_191507870.1
15	WP_129009770.1	40	MBR5981728.1
16	MTK10086.1	41	WP_215633330.1
17	WP_106009325.1	42	MBP3327663.1
18	HBB29922.1	43	WP_152889336.1
19	WP_216437565.1	44	WP_052356672.1
20	WP_090039956.1	LmACR	Q8Y7U1
21	WP_083963349.1	PtACDH	A0A178TYF6
22	WP_012101452.1	StEutE	P41793
23	WP_122646076.1	RpPduP	Q21A49
24	WP_070791575.1	BmACDH	I3E349
25	WP_010715224.1	CbAld	Q716S8

[0104] Table 3. List of enzymes used for the conversion of intermediate to 1,4-butanediol

RX/Enz	Gene	GenBank Accession Number
DDR/GDHt	KpDhaB123	Q59476, O08505, Q59475
DDR/GDHt	CbDhaB12	Q8GEZ8, Q8GEZ7
DDR/GDHt	CfDhaBCE	P45514, A8CH91, A8CH95
DDR/GDHt	KoPddABC	Q59470, Q59471, Q59472
DDR/GDHt	EcPddAB	A0A828U7A4, A0A828U8K0
DDR/GDHt	CdDhaB1	ACI39932
DDR/GDHt	ChDhaB	ZP_03292457.1
DDR/GDHt	CmDhaB	ZP_03706520.1
DDR/GDHt	CnPfl2	WP_011721311.1
DDR/GDHt	CpPfl2	WP_012199200.1
DDR/GDHt	ClDhaB	ABW38375
DDR/GDHt	CtDhaB	WP_011099190.1
DDR/GDHt	DhPfl	WP_015943317.1
DDR/GDHt	DsPfl2	ZP_03467192.1
DDR/GDHt	EpDha	ZP_02692139.1
DDR/GDHt	EcPflD	NP_418386
DDR/GDHt	EhPfl2	ZP_03717880.1

DDR/GDHt	PcDhaB	YP_356813.1
DDR/GDHt	RpPfl2	WP_011471631.1
DDR/GDHt	RrPfl2	WP_011388658.1
DDR/GDHt	RiDhaB	ABC25539
DDR/GDHt	RgDhaB	ZP_02040253.1
DDR/GDHt	RoDhaB	ZP_01962384.1
DDR/GDHt	SePflD	ZP_02701301.1
DDR/GDHt	StPflD	NP_462994
DDR/GDHt	GamPfl2	WP_011787819.1
DDR/GDHt	SfPflD	NP_709751
DDR/GDHt	SaPfl2	ZP_00789518.1
DDR/GDHt	VsPflD	ZP_01867938.1
ADH	EcFucO	P0A9S1
ADH	EcAdhE	P0A9Q7
ADH	CaAhdE2	Q9ANR5
ADH	ScALD5	P40047
ADH	EcYqhD	Q46856
ADH	EcYahK	P75691
ADH	TrGLD1	Q0GYU5

[0105] Note: DDR: diol dehydratase; GDHt: glycerol dehydratase; ADH: alcohol dehydrogenase.

[0106] Table 4. List of enzymes used for the interconversion of one-carbon compounds to generate formyl-CoA

Reaction	Gene	GenBank Accession Number
sMMO	mmoXYBZCD	P18797
pMMO	pmoA1A2B1B2	G1UBD1 Q607G3
MDH	BmMDH2	I3E2P9
	CnMDH2	F8GNE5
	BsMDH	P42327
	MeMxaF-MxaI	P16027, P14775
AOD	CbAOD1	Q00922
	PaMOX	P04841
	PpAOX1	P04842
	PpAOX2	F2R038
	PmMOD1	Q9UVU2
	PmMOD2	Q9UVU1
CTA	CbCTA	Q96VB8

	PaPXP9	P30263
	PpCTA1	C4R2S1
	PmCTA1	E0D5F4
ACS	EcACS	P27550
	BsACS	P39062
	StACSstab	Q8ZKF6
	MhACS	F2NQX2
	ArACS	A0A0Q7JEV7

[0107] Note: sMMO: soluble methane monooxygenase; pMMO: Particulate methane monooxygenase; MDH: methanol dehydrogenase; AOD: alcohol oxidase; ACS: acyl-CoA synthase.

[0108] Table 5. List of acyl-CoA transferases (ACT) variants used for the interconversion of one-carbon compounds to generate formyl-CoA

ACT#	GenBank Accession Number	ACT#	GenBank Accession Number
1	NBW24427.1	33	MBW7889249.1
2	HBE84973.1	34	MBK7676927.1
3	WP_132821656.1	35	MBV8105822.1
4	WP_220287672.1	36	MBP7764230.1
5	WP_121965416.1	37	MSO92582.1
6	XP_014530103.1	38	MBE9574050.1
7	2OAS_1	39	MBX9950024.1
8	WP_084235291.1	40	AEK61848.1
9	MBS6366228.1	41	MBL8096225.1
10	MBR5999861.1	42	OPZ27283.1
11	3D3U_1	43	RYD04206.1
12	WP_073092544.1	44	MBQ9059638.1
13	MBR0090292.1	45	2G39_1
14	MBU4349155.1	46	2NVV_1
15	NLU48536.1	47	MBQ9530926.1
16	3EH7_1	48	4EU3_1
17	MBS0639490.1	49	KAF4531260.1
18	WP_194298948.1	50	2HJ0_1
19	3UBM_A	51	SJZ60628.1
20	WP_203555095.1	52	ALP92681.1
21	MBC7087538.1	53	GFX41336.1
22	WP_191390389.1	54	5VIT_1
23	MBR0127170.1	55	SDR52074.1

24	WP_206582952.1	56	MBF0160244.1
25	MBK5252043.1	57	MBF0445994.1
26	MBR2778738.1	58	HGX18505.1
27	MBF8291010.1	59	MBK9387745.1
28	RLI25587.1	60	MBF0310027.1
29	HHW62298.1	61	RKY20198.1
30	NWF83872.1	62	NYH33089.1
31	NUN70050.1	63	Q9RM86
32	MBU2447274.1	64	O06644

[0109] Table 6. List of pairs of acyl-CoA kinases (ACK) and phosphoacyltransferases (PTA) used for the interconversion of one-carbon compounds to generate formyl-CoA

JGIK #	GenBank Accession Number		JGIK #	GenBank Accession Number	
	ACK	PTA		ACK	PTA
1	AKJ38693.1	AKJ38694.1	21	MBU2064158.1	MBU2064159.1
2	BCV24779.1	BCV24778.1	22	MBU2501086.1	MBU2501087.1
3	EDM85332.1	EDM85331.1	23	NLA96479.1	NLA96478.1
4	GFI65571.1	GFI65570.1	24	NLI62094.1	NLI62095.1
5	HBG22385.1	HBG22386.1	25	NLM93282.1	NLM93283.1
6	HFV10353.1	HFV10352.1	26	NMA59030.1	NMA59029.1
7	HGG13858.1	HGG13857.1	27	OGI05344.1	OGI05345.1
8	HIX51076.1	HIX51075.1	28	OHB58473.1	OHB58472.1
9	KXK65140.1	KXK65141.1	29	PWM39673.1	PWM39672.1
10	KXL51791.1	KXL51790.1	30	TKJ47541.1	TKJ47542.1
11	MBE5816841.1	MBE5816840.1	31	WP_022744670.1	WP_022744669.1
12	MBE6451999.1	MBE6451998.1	32	WP_023275423.1	WP_023275424.1
13	MBF0205569.1	MBF0205570.1	33	WP_076546120.1	WP_076546119.1
14	MBI4335478.1	MBI4335477.1	34	WP_078810629.1	WP_078810628.1
15	MBN1633386.1	MBN1633385.1	35	WP_099343330.1	WP_099343331.1
16	MBR2784446.1	MBR2784445.1	36	WP_106012460.1	WP_106012461.1
17	MBR3082232.1	MBR3082233.1	37	AAA72042.1	AAA72041.1
18	MBS5449595.1	MBS5449596.1	38	BAG33697.1	BAG33698.1
19	MBS6942200.1	MBS6942201.1	39	A0A0J8DB00	A0A0J8D6J2

20	MBU0667180. 1	MBU0667181. 1	40	P0A6A3	P0A9M8
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[0110] Various embodiments have been described herein with reference to the accompanying drawings. It will, however, be evident that various modifications and changes may be made thereto, and additional embodiments may be implemented, without departing from the broader scope of the invention as set forth in the claims that follow. Further, other embodiments will be apparent to those skilled in the art from consideration of the specification and practice of one or more embodiments of the invention disclosed herein. It is intended, therefore, that this application and the examples herein be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following listing of exemplary claims.