

DESCRIPTION

TITLE OF THE INVENTION: PRODUCTION OF METHIONINE VIA CONDENSATION OF C1-DERIVED FORMYL-COA WITH 3-HYDROXYPROPIONALDEHYDE DERIVED FROM VARIOUS SUBSTRATES

TECHNICAL FIELD

[0001] The present disclosure generally relates to a method for producing methionine, in particular, a method for producing methionine via condensation of C1-derived formyl-CoA with 3-hydroxypropionaldehyde derived from various substrates. The present disclosure also relates to genetically modified microorganism used for producing methionine.

SUMMARY OF THE INVENTION

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

[0003] Embodiment 1. A method for the production of 2,4-dihydroxybutyryl-CoA comprising 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.

[0004] Embodiment 2. The method of Embodiment 1, wherein the TPP-dependent enzyme is 2-hydroxyacyl-CoA synthase or 2-hydroxyacyl-CoA lyase.

[0005] Embodiment 3. The method of Embodiment 2, 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	or .
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			

[0006] Embodiment 4. The method of Embodiment 1, further comprising generating formyl-CoA by contacting a one-carbon (C1) substrate selected from formaldehyde or formate with an enzyme.

[0007] Embodiment 5. The method of Embodiment 4, wherein the C1 substrate is formaldehyde, and the enzyme is an acyl-CoA reductase.

[0008] Embodiment 6. The method of Embodiment 4, wherein the C1 substrate 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.

[0009] Embodiment 7. The method of Embodiment 5, 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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[0010] Embodiment 8. The method of Embodiment 6, wherein the formate kinase and the phosphate formyltransferase pair is selected from:

JGI K#	GenBank Accession Number		JGI K#	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

or
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[0011] Embodiment 9. The method of Embodiment 6, 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
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

or
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[0012] Embodiment 10. The method of Embodiment 6, wherein the acyl-CoA synthase is selected from:

Gene	GeneBank
EcACS	P27550
BsACS	P39062
StACSstab	Q8ZKF6_PROSS
MhACS	F2NQX2
ArACS	A0A0Q7JEV7

or
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[0013] Embodiment 11. The method of Embodiment 5, further comprising generating formaldehyde from methanol by contacting methanol with a methanol dehydrogenase or methanol oxidase.

[0014] Embodiment 12. The method of Embodiment 11, wherein the methanol dehydrogenase or methanol oxidase is selected from:

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

[0015] Embodiment 13. The method of Embodiment 11 or 12, wherein further comprising generating methanol from methane by contacting methane with a methane monooxygenase.

[0016] Embodiment 14. The method of Embodiment 13, wherein the methane monooxygenases is selected from:

Gene	GeneBank	
mmoXYBZCD	P18797	or
pmoA1A2B1B2	G1UBD1 Q607G3	.

[0017] Embodiment 15. The method of any one of Embodiments 1 to 14, further comprising generating 3-hydroxypropionaldehyde from glycerol.

[0018] Embodiment 16. The method of any one of Embodiments 1 to 14, further comprising generating 3-hydroxypropionaldehyde from ethylene glycol and C1 compound.

[0019] Embodiment 17. The method of Embodiment 16, further comprising generating glycolaldehyde from ethylene glycol by contacting ethylene glycol 1 with an alcohol dehydrogenase.

[0020] Embodiment 18. The method of Embodiment 17, wherein the alcohol dehydrogenase is selected from:

YqhD	NP_417484
YahK	P75691
FucO	
YjgB	P27250

[0021] Embodiment 19. The method of Embodiment 16-18, further comprising generating glyceryl-CoA from glycolaldehyde and formyl-CoA by a TPP-dependent enzyme selected from 2-hydroxyacyl-CoA synthase, 2-hydroxyacyl-CoA lyase, oxalyl-CoA decarboxylase or benzaldehyde lyase.

[0022] Embodiment 20. The method of Embodiment 16-19, further comprising generating glyceraldehyde from glyceryl-CoA by acyl-CoA reductase.

[0023] Embodiment 21. The method of Embodiment 16-20, further comprising generating glycerol from glyceraldehyde by alcohol dehydrogenase.

[0024] Embodiment 22. The method of Embodiment 21, wherein the alcohol dehydrogenase is selected from:

YqhD	NP_417484
YahK	P75691
Ybbo	Q46856
YjgB	P27250
AKR1B1	AAA51713.1
GLD1	ABD83952.1

[0025] Embodiment 23. The method of any one of Embodiments 1 to 16, further comprising generating 3-hydroxypropionaldehyde from a C1 compound including but not limited to formate, formaldehyde, and methanol.

[0026] Embodiment 24. The method of any one of Embodiments 1 to 23, further comprising generating formyl-CoA from interconversion of C1 compounds.

[0027] Embodiment 25. The method of any one of Embodiments 1 to 24, further comprising generating formaldehyde from interconversion of C1 compounds.

[0028] Embodiment 26. The method of any one of Embodiments 1 to 25, further comprising generating glycolyl-CoA from formaldehyde and formyl-CoA by a TPP-dependent enzyme selected from 2-hydroxyacyl-CoA synthase, 2-hydroxyacyl-CoA lyase, oxalyl-CoA decarboxylase or benzaldehyde lyase.

[0029] Embodiment 27. The method of any one of Embodiments 1 to 26, further comprising generating glycolaldehyde from glycolyl-CoA by acyl-CoA reductase.

[0030] Embodiment 28. The method of any one of Embodiments 1 to 27, further comprising generating glyceryl-CoA from glycolaldehyde by a TPP-dependent enzyme selected from 2-hydroxyacyl-CoA synthase, 2-hydroxyacyl-CoA lyase, oxalyl-CoA decarboxylase or benzaldehyde lyase.

[0031] Embodiment 29. The method of any one of Embodiments 1 to 28, further comprising generating glyceraldehyde from glyceryl-CoA by acyl-CoA reductase.

[0032] Embodiment 30. The method of any one of Embodiments 1 to 29, further comprising generating glycerol from glyceraldehyde by alcohol dehydrogenase.

[0033] Embodiment 31. A method for the production of 3-hydroxypropionaldehyde comprising converting glycerol to 3-hydroxypropionaldehyde by contacting glycerol with glycerol dehydratase.

[0034] Embodiment 32. The method of Embodiment 31, wherein the glycerol dehydratase is selected from:

DhaB123	Q59476, O08505, Q59475	or .
DhaB12	Q8GEZ8, Q8GEZ7	
DhaBCE	P45514, A8CH91, A8CH95	

[0035] Embodiment 33. A method for the production of 2,4-dihydroxybutyric acid comprising:

- (a) providing 2,4-dihydroxybutyryl-CoA obtained by the method of any one of Embodiments 1-29; and
- (b) converting 2,4-dihydroxybutyryl-CoA to 2,4-dihydroxybutyric acid by contacting 2,4-dihydroxybutyryl-CoA with any one of (i) and (iii):
 - (i) a thioesterase or an acyl-CoA transferase catalyzing the conversion of 2,4-dihydroxybutyryl-CoA to 2,4-dihydroxybutyric acid;
 - (ii) a phosphotransacylase catalyzing the conversion of 2,4-dihydroxybutyryl-CoA to 2,4-dihydroxybutyryl-phosphate and a carboxylate kinase catalyzing the conversion of 2,4-dihydroxybutyryl-phosphate to 2,4-dihydroxybutyric acid; or
 - (iii) an acyl-CoA reductase catalyzing the conversion of 2,4-dihydroxybutyryl-CoA to 2,4-dihydroxybutanal and an aldehyde dehydrogenase catalyzing the conversion of 2,4-dihydroxybutanal to 2,4-dihydroxybutyric acid.

[0036] Embodiment 34. The method of any one of Embodiments 20, 27, and 33, 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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[0037] Embodiment 35. The method of Embodiment 33, wherein the aldehyde dehydrogenase is:

AldA	P25553
AldB	P37685

[0038] Embodiment 36. A method for the production of 2-keto-4-hydroxybutyric acid comprising:

- (a) providing 2,4-dihydroxybutyric acid by the method of any one of Embodiments 33 to 35;
- and
- (b) converting 2,4-dihydroxy-butylric acid to 2-keto-4-hydroxybutyric acid by contacting 2,4-dihydroxybutyric acid with a 2-hydroxy acid dehydrogenase.

[0039] Embodiment 37. The method of Embodiment 34, wherein the 2-hydroxy acid dehydrogenase is selected from:

Gene	GeneBank
CtBP1	U37408
2HADH	A0A8V0XVZ4
EcMdh-5Q	P61889 (5Q)
LlLdhA(Q85C)	Q01462
RmLdh	P13491
LlPanE	Q9CFY8
LldD	P33232
GLO2	Q9LRS0
dld	P06149

or
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[0040] Embodiment 38. A method for the production of 2-keto-4-(methylthio)butyric acid comprising:

- (a) providing 2-keto-4-hydroxybutyric acid by the method of Embodiment 36 or 37; and
- (b) converting 2-keto-4-hydroxybutyric acid to 2-keto-4-(methylthio)butyric acid by chemically condensing 2-keto-4-hydroxybutyric acid with methanethiol (CH_3SH) or sodium or potassium salt thereof (i.e., sodium methanethiolate (CH_3NaS) or potassium methanethiolate (CH_3KS)).

[0041] Embodiment 39. A method for the production of L-methionine or D-methionine comprising:

- (a) providing 2-keto-4-(methylthio)butyric acid by the method of Embodiment 38; and
- (b) converting 2-keto-4-(methylthio)butyric acid to L-methionine or D-methionine by contacting 2-keto-4-(methylthio)butyric acid with an enzyme selected from an amino acid dehydrogenase or a methionine/homoserine aminotransferase.

[0042] Embodiment 40. The method of Embodiment 39, wherein the amino acid dehydrogenase is selected from:

Gene	GeneBank
Bcd	M4KZ55
dadA1	Q9HTQ0
dadA	M1K4N6
dadA	B4EXU2
MtAld	P9WQB1
BsAld	Q08352

or
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[0043] Embodiment 41. The method of Embodiment 38, wherein the methionine/homoserine aminotransferase is selected from:

Gene	GeneBank	or .
YbdL	A0A378BX14	
YbdL	P77806	
Aro8	P53090	
BCAT	Q9LE06	.

[0044] Embodiment 42. The method of any one of Embodiments 1 to 41, wherein the enzyme used in the method is isolated from a microorganism.

[0045] Embodiment 43. The method of any one of Embodiments 1 to 41, wherein the enzyme used in the method is contained in a microorganism.

[0046] Embodiment 44. A genetically modified microorganism providing 2,4-dihydroxybutyryl-CoA by the method of any one of Embodiments 1 to 30.

[0047] Embodiment 45. The microorganism of Embodiment 44, further providing 2,4-dihydroxybutyric acid by the method of any one of Embodiments 33 to 35.

[0048] Embodiment 46. The microorganism of Embodiment 44 or 45, further providing 2-keto-4-hydroxybutyric acid by the method of Embodiment 36 or 37.

[0049] Embodiment 47. The microorganism of any one of Embodiments 44 to 46, further providing 2-keto-4-(methylthio)butyric acid by the method of Embodiment 38.

[0050] Embodiment 48. The microorganism of any one of Embodiments 44 to 47, further providing L-methionine or D-methionine by the method of any one of Embodiments 39 to 41.

[0051] Embodiment 49. The microorganism of Embodiments 44 to 48, wherein the microorganism is selected from the group consisting of bacteria, yeast and fungi.

[0052] Embodiment 50. The microorganism of Embodiments 44 to 49, wherein the microorganism is bacteria, yeast or fungi, including but not limited to *Escherichia sp.*, *Bacillus sp.*, *Pseudomonas sp.*, *Corynebacterium sp.*, *Zyomonas 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.*, *Trichoderma sp.*

[0053] 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.

[0054] The present disclosure is illustrated by the following non-limiting examples.

[0055] 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

[0056] 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.

[0057] Figure 1 is a scheme illustrating production of desmeninol, homoserine and methionine from condensation of 3-hydroxypropionaldehyde and C1-derived formyl-CoA. MMO: methane monooxygenase; MDH: methanol dehydrogenase; 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; 2HADH: 2-hydroxyacid dehydrogenase; AADH: amino acid dehydrogenase; MAT: methionine aminotransferase; HAT: homoserine aminotransferase.

[0058] Figure 2 is a scheme illustrating production of 3-hydroxypropionaldehyde from glycerol, ethylene glycol and C1 compounds through the formyl-CoA elongation (FORCE) pathway. MMO: methane monooxygenase; MDH: methanol dehydrogenase; 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; GDHt: Glycerol dehydratase; ADH: alcohol dehydrogenase.

[0059] Figure 3a is a scheme illustrating interconversion of C1 compounds, Figure 3b is a scheme illustrating GSH and MSH-dependent oxidation of formaldehyde to formate, and Figure 3c is a scheme illustrating H₄F and H₄MPT-dependent oxidation of formaldehyde to formate. 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 enzyme; MCH: methenyl-H₄MPT cyclohydrolase.

[0060] The same reference numbers will be used throughout the drawings to refer to the same or like parts.

DETAILED DESCRIPTION OF THE INVENTION

[0061] 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.

[0062] 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.

[0063] The compounds of present disclosure can comprise one or more asymmetric centers, and thus can exist in various stereoisomeric forms, e.g., enantiomers and/or diastereomers. Thus, the compounds of present disclosure and compositions thereof may be in the form of an individual enantiomer, diastereomer or geometric isomer, or may be in the form of a mixture of stereoisomers. In certain embodiments, the compounds of the present disclosure are enantiopure compounds. In certain embodiments, mixtures of enantiomers or diastereomers are provided. A stereoisomer may, in some embodiments, be provided substantially free of one or more corresponding stereoisomers, and may also be referred to as “stereochemically enriched”.

[0064] Where a particular enantiomer is preferred, it may, in some embodiments be provided substantially free of the opposite enantiomer, and may also be referred to as “optically enriched”. “Optically enriched”, as used herein, means that the compound is made up of a significantly greater proportion of one enantiomer. In certain embodiments, the compound is made up of at least about 90% by weight of a preferred enantiomer. In other embodiments, the compound is made up of at least about 95%, 98%, or 99% by weight of a preferred enantiomer.

[0065] The terms “2,4-dihydroxybutyric acid” and “2,4-dihydroxybutanoic acid” are used interchangeably. The terms “2-keto-4-hydroxybutyric acid” and “4-hydroxy-2-oxobutanoic acid” are used interchangeably. The terms “2-keto-4-(methylthio)butyric acid” and “4-(methylthio)-2-oxobutanoic acid” are used interchangeably.

[0066] EXAMPLES

[0067] EXAMPLE 1: PRODUCTION OF METHIONINE THROUGH CONDENSATION OF 3-HYDROXYPROPIONALDEHYDE AND FORMYL-COA

[0068] This Example demonstrates the implementation of methionine production through the C1+Bio platform by condensation of 3-hydroxypropionaldehyde and formyl-CoA and followed by derivation to methionine (**Figure 1** below). The condensation can be catalyzed by HACS (e.g., HACS set forth in **Table 1** below), including but not limited to beach sand metagenome BsmHACS (GeneBank accession: HAK63664.1), DhcHACS from *Dehalococcoidia bacterium* (GeneBank accession: PWB41796.1), CfhHACS from *Chloroflexi bacterium* (GeneBank accession: PKN81274.1), to generate 2,4-dihydroxybutyryl-CoA. 2,4-dihydroxybutyryl-CoA is spontaneously converted to 2-hydroxybutyrolactone. Alternatively, 2,4-dihydroxybutyryl-CoA is further converted to 2,4-dihydroxybutyranic acid through the action of a thioesterase (such as TesB from *E. coli* or *Pseudomonas putida* (McMahon, M.D. and Prather, K.L.J. Appl. Environ. Microbiol. 2014, 80:1042-1050)), CoA transferase (ACT, e.g., set forth in **Table 5** below), or phosphotransacylase-acid kinase (PTA-ACK, e.g., set forth in **Table 6** below). Alternatively, 2,4-dihydroxybutyryl-CoA is reduced to 2,4-dihydroxybutanal by acyl-CoA reductase (ACR) from *Salmonella typhimurium* (StEutE, GeneBank accession: P41793) or other ACR variants (e.g., ACR set forth in Table 2 below), followed by oxidation of 2,4-dihydroxybutanal to 2,4-dihydroxybutanoic acid with the help of aldehyde dehydrogenase (ALDH set forth in **Table 3**

below). And 2,4-dihydroxybutanoic acid is lactonized to 2-hydroxybutyrolactone by adjusting the pH to lower than 6.

[0069] 2-hydroxybutyrolactone can be chemically condensed with methanethiol or sodium or potassium salt thereof (CH_3SH , CH_3NaS or CH_3KS) to form desmeninol. Desmeninol could convert to 4-(methylthio)-2-oxobutanoic acid with the help of 2-hydroxy acid dehydrogenase (2HADH). Alternatively, 2-hydroxybutyrolactone can be converted to 2-ketobutyrolactone with the help of 2HADH, and 2-ketobutyrolactone together with CH_3SH can be chemically convert to 4-(methylthio)-2-oxobutanoic acid. 4-(methylthio)-2-oxobutanoic acid can be further converted to methionine which can be catalyzed by amino acid dehydrogenase (AADH) (Nitta et al., J Bacteriol. 1974; 117(2): 588–592) or methionine aminotransferase (MAT) (Venos et al., BMC Microbiol, 2004; 4, 39). (Fig. 1 below; enzymes set forth in **Table 3** below)

[0070] In parallel, 2-hydroxybutyrolactone can be reduced to 2-ketobutyrolactone by 2HADH and then generate homoserine lactone which can be catalyzed by amino acid dehydrogenase (AADH) (Nitta et al., J Bacteriol. 1974; 117(2): 588–592) or homoserine aminotransferase (HAT) (Venos et al., BMC Microbiol, 2004; 4, 39). Homoserine lactone can be converted to methionine chemically together with CH_3SH . Homoserine lactone can be converted to homoserine as well by adjusting the pH to higher than pH 8. (Fig. 1)

[0071] Alternatively, 2,4-dihydroxybutanoic acid is firstly converted to 4-hydroxy-2-oxobutanoic acid by 2HADH, followed by adding the amino group to 4-hydroxy-2-oxobutanoic acid to generate homoserine. Homoserine can be further chemically converted to methionine (Fig. 1).

EXAMPLE 2: PRODUCTION OF 3-HYDROXYPROPIONALDEHYDE FROM GLYCEROL, ETHYLENE GLYCOL AND ONE-CARBON COMPOUNDS

[0072] This example demonstrates the 3-hydroxypropionaldehyde production from different feedstocks, including but not limited to glycerol (a C3 compound), ethylene glycol (a C2 compound) and methanol (a C1 compound). When 3-hydroxypropionaldehyde is produced, it can be further condensed with formyl-CoA generated from C1 compounds to form 2,4-dihydroxybutyryl-CoA and finally converted to methionine as presented in **Fig. 1**.

[0073] When 3-hydroxypropionaldehyde is produced from glycerol. Glycerol dehydratase (GDHt) needs to be overexpressed for 3-hydroxypropionaldehyde production from glycerol (**Fig. 2**).

[0074] When 3-hydroxypropionaldehyde is produced from ethylene glycol, two iterative condensations are needed for methionine production (**Fig. 2**). Ethylene glycol is firstly converted to glycolaldehyde by *E. coli* FucO. Glycolaldehyde can be condensed with formyl-CoA to generate glyceryl-CoA, a reaction catalyzed by HACS, including CfhHACS from *Chloroflexi bacterium* (GeneBank accession: PKN81274.1), RbhHACS from *Rhodocyclaceae* bacterium (GeneBank accession: MBL8483477.1), PdsHACS from *Pseudonocardia sediminis* (GeneBank accession: WP_130292058.1) or other HACS variants (e.g., HACS set forth in **Table 1** below). Glyceryl-CoA can be reduced to glyceraldehyde by acyl-CoA reductase (ACR) from *Salmonella typhimurium* (StEutE, GeneBank accession: P41793) or other ACR variants (e.g., ACR set forth in **Table 2** below). Glyceraldehyde was further converted to glycerol by glyceraldehyde reductase (ADH), such as *yqhD*, *yahK* from *E. coli*, GLD1 from *Trichoderma reesei* (e.g., enzymes set forth in **Table 3** below). Glycerol can be converted to 3-hydroxypropionaldehyde as mentioned above by glycerol dehydratase GDHt, *dhaB123* from *Klebsiella pneumonia* or other microorganisms (e.g., GDHt set forth in **Table 3** below), and then further convert to methionine through the route described in example 1.

[0075] When 3-hydroxypropionaldehyde is produced from methanol or other C1 compounds, one more iterative condensation is needed to generate glycolaldehyde for methionine production compare to using ethylene glycol as co-substrate (**Fig. 2**). Methanol or other C1 compounds were firstly converted to formaldehyde and formyl-CoA through the interconversion module (**Fig. 3**). Formaldehyde and formyl-CoA condensed together to generate glycolyl-CoA by HACS , including CfhHACS from *Chloroflexi bacterium* (GeneBank accession: PKN81274.1), RbhHACS from *Rhodocyclaceae* bacterium (GeneBank accession: MBL8483477.1), PdsHACS from *Pseudonocardia sediminis* (GeneBank accession: WP_130292058.1) or other HACS variants (e.g., HACS set forth in **Table 1** below), and further converted to glycolaldehyde with ACR variants from *Salmonella typhimurium* (StEutE, GeneBank accession: P41793) or other ACR variants (e.g., ACR set forth in **Table 2** below). And glycolaldehyde can be used as a substrate for next round condensation with formyl-CoA to generate glyceryl-CoA that catalyzed by above mentioned HACS variants (e.g., HACS set forth in **Table 1** below). Glyceryl-CoA can be reduced to glyceraldehyde by acyl-CoA reductase (ACR) from *Salmonella typhimurium* (StEutE, GeneBank accession: P41793) or other ACR variants (e.g., ACR set forth in **Table 2** below). Glyceraldehyde was further converted to glycerol by glyceraldehyde reductase (ADH), such as

yqhD, *yahK* from *E. coli*, GLD1 from *Trichoderma reesei* (e.g., enzyme set forth in **Table 3** below). Glycerol can be converted to 3-hydroxypropionaldehyde as mentioned above by glycerol dehydratase (GDHt), and then further convert to methionine through the route described in example 1.

[0076] EXAMPLE 3. INTERCONVERSION OF ONE-CARBON COMPOUNDS TO GENERATE FORMYL-COA

[0077] Formyl-CoA can be provided through the interconversion of C1 compounds including but not limited to methane, methanol, formaldehyde, formate (**Figure 3a** 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 used to detoxify of hydrogen peroxide (e.g., enzymes set forth in **Table 4** below).

[0078] 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. (Fig. 3b) 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 FTFL (Fig. 3c).

[0079] 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 4-methylthio-2-hydroxy butyryl-CoA, 2,4-dihydroxy butyryl-CoA, acetyl-CoA or succinyl-CoA to formate. Phosphotransacylase-formate kinase (PTA-FOK, e.g., set forth in **Table 6** below) pair catalyze 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. (Fig. 3a)

[0080] EXAMPLE 4: OVERVIEW OF METHODS USED FOR GENETIC MANIPULATION

[0081] 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.

[0082] 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).

[0083] 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.

[0084] EXAMPLE 5: FERMENTATION CONDITIONS USED FOR IN VIVO PRODUCTION OF METHIONINE

[0085] MOPS minimal medium (Neidhardt et al. J. Bacteriol.119736-47 (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 µM 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 µg/mL carbenicillin, 50 µg/mL spectinomycin and 50 µg/mL kanamycin) are included when appropriate. All chemicals are obtained from Fisher Scientific Co. (Pittsburg, PA) and Sigma-Aldrich Co. (St. Louis, MO).

[0086] 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 completely 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.

[0087] 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.

[0088] EXAMPLE 6. HPLC ANALYSIS OF METHIONINE

[0089] The supernatant aliquots of 0.1 mL transferred to 15 mL tube, mixed with 0.2 mL of acetonitrile, 0.1 mL of 0.5 mM NaHCO₃ and 0.1 mL of 2,4-Dinitrofluorobenzene (DNFB) solution (0.2 mL DNFB add acetonitrile to 10 mL), tighten the cap and vigorously vortex for 30 seconds before doing derivatization. The derivatization reaction was carried out on a water bath (60 °C) for 20 min in the dark. After cooling in ice-cold water, 1 mL of 0.5 M phosphate buffer solution was added to terminate the reaction. Another 1 mL acetonitrile and 7.5 mL MiniQ water was added. The resulting solution of DNFB derivatives was then briefly vortexed again and filtered through a 0.22 µm nylon filter into auto-sampler vials for HPLC analysis.

[0090] HPLC analysis is conducted on a Waters 2695 system equipped with 996 photodiode array detector and a Phenomenex Luna C18 column (250mm*4.6mm*5µm). Column temperature was maintained at 30 °C, detector was set at 360 nm, and the injection volume was 10 µL. The mobile phase consisting of 0.1% acetic acid and 0.1% trimethylamine in MiniQ water (A), and acetonitrile (B). The separation was achieved using the following gradient conditions with a flow rate of 0.8

mL/min: 0 min, 30% acetonitrile (B); 15 min, 85% acetonitrile (B); 18 min, 85% acetonitrile (B); 18.01 min, 30% acetonitrile (B); 30 min, 30% acetonitrile (B).

[0091] Table 1. List of 2-hydroxyacyl-CoA synthase (HACS) used for the condensation of methional and formyl-CoA

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		

[0092] 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

[0093] Table 3. List of enzyme used for the conversion of intermediate to methionine

RX/Enz	Gene	GeneBank
2HADH	CtBP1	U37408
2HADH	2HADH	A0A8V0XVZ4
2HADH	EcMdh-5Q	P61889 (5Q)
2HADH	LlLdhA(Q85C)	Q01462
2HADH	RmLdh	P13491
2HADH	LlPanE	Q9CFY8
2HADH	LldD	P33232
2HADH	GLO2	Q9LRS0
2HADH	dld	P06149
GDHt	DhaB123	Q59476, O08505, Q59475
GDHt	DhaB12	Q8GEZ8, Q8GEZ7
GDHt	DhaBCE	P45514, A8CH91, A8CH95
ADH	YqhD	NP_417484
ADH	YahK	P75691
ADH	Ybbo	Q46856
ADH	YjgB	P27250
ADH	AKR1B1	AAA51713.1
ADH	GLD1	ABD83952.1
ALDH	AldA	P25553
ALDH	AldB	P37685
AADH	Bcd	M4KZ55
AADH	DadA1	Q9HTQ0
AADH	DadA	M1K4N6
AADH	DadA	B4EXU2
AADH	MtAld	P9WQB1
AADH	BsAld	Q08352
MAT	YbdL	A0A378BX14
MAT	YbdL	P77806
MAT	Aro8	P53090
MAT	BCAT	Q9LE06

[0094] Note: 2HADH: 2-hydroxyacid dehydrogenase; GDHt: glycerol dehydratase; ADH: alcohol dehydrogenase; ALDH: aldehyde dehydrogenase; AADH: amino acid dehydrogenase; MAT: methionine aminotransferase

[0095] Table 4. List of enzymes used for the interconversion of one-carbon compounds to generate formyl-CoA

Reaction	Gene	GeneBank 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_PROSS
	MhACS	F2NQX2
	ArACS	A0A0Q7JEV7

[0096] Note: sMMO: soluble methane monooxygenase; pMMO: Particulate methane monooxygenase; MDH: methanol dehydrogenase; AOD: oxygen-dependent alcohol oxidase; CTA: catalase; ALDH: aldehyde dehydrogenase; ACS: acyl-CoA synthase.

[0097] 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
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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

[0098] Table 6. List of List of acyl-CoA kinases (ACK) and phosphoacyltransferases (PTA) variants (JGIK)variants 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

[0099] 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.

Claims

- Claim 1: A genetically modified microorganism for converting 3-hydroxypropionaldehyde and formyl-CoA to 2,4-dihydroxybutyryl-CoA, comprising an overexpressed TPP-dependent enzyme selected from a 2-hydroxyacyl-CoA synthase, a 2-hydroxyacyl-CoA lyase, an oxalyl-CoA decarboxylase or a benzaldehyde lyase.
- Claim 2: The genetically modified microorganism of claim 1, further comprising an overexpressed enzyme catalyzing the conversion of 2,4-dihydroxybutyryl-CoA to 2,4-dihydroxybutyric acid, wherein the overexpressed enzyme is selected from thioesterase, acyl-CoA transferase, a phosphotransacylase and a carboxylate kinase or an acyl-CoA reductase and an aldehyde dehydrogenase.
- Claim 3: The genetically modified microorganism of claim 2, further comprising an overexpressed 2-hydroxy acid dehydrogenase catalyzing the conversion of 2,4-dihydroxybutyric acid to 2-keto-4-hydroxybutyric acid.
- Claim 4: A genetically modified microorganism for converting 2-keto-4-(methylthio)butyric acid to L-methionine or D-methionine, comprising an overexpressed amino acid dehydrogenase, or methionine aminotransferase.
- Claim 5: The genetically modified microorganism of claim 4, further comprising an overexpressed amino acid dehydrogenase catalyzing the conversion of 2-keto-4-hydroxybutyric acid to L-homoserine or D-homoserine.
- Claim 6: The genetically modified microorganism of claim 4, further comprising an overexpressed aminotransferase catalyzing the conversion of 2-keto-4-hydroxybutyric acid to L-homoserine or D-homoserine.
- Claim 7: The genetically modified microorganisms of any one of claims 1-6, further comprising an overexpressed enzyme selected from the group consisting of:
- (i) an acylating formaldehyde dehydrogenase converting formaldehyde to formyl-CoA;
 - (ii) a formate kinase converting formate to formyl-phosphate and a phosphate formyltransferase converting formyl-phosphate to formyl-CoA;
 - (iii) an acyl-CoA transferase converting formate to formyl-CoA; and

(iv) an acyl-CoA synthase converting formate to formyl-CoA.

Claim 8: The genetically modified microorganisms of any one of claims 1-7, capable of converting 3-hydroxypropionaldehyde and a C1 substrate selected from methane, methanol, formaldehyde or formate to a product selected from 2,4-dihydroxybutyric acid, hydroxybutyrolactone, desmeninol, 2-keto-4-(methylthio)butyric acid, L-methionine or D-methionine.

Claim 9: The genetically modified microorganisms of any one of claims 1-8, wherein the microorganisms are selected from the group consisting of bacteria, yeast and fungi.

Claim 10: The genetically modified microorganisms of any one of claims 1-9, wherein the microorganisms are *Escherichia sp.*, *Bacillus sp.*, *Pseudomonas sp.*, *Corynebacterium sp.*, *Zymonas sp.*, *Clostridium sp.*, *Streptococcus sp.*, *Rhodococcus sp.*, *Geobacillus sp.*, *Saccharomyces sp.*, *Pichia sp.*, *Yarrowia sp.*, *Methylorubrum sp.*, *Candida sp.*, *Kluyveromyces sp.*, *Aspergillus sp.*, *Penicillium sp.*, *Rhizopus sp.* *Trichoderma sp.*.

Claim 11: A method for the production of 2,4-dihydroxybutyryl-CoA comprising 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.

Claim 12: A method for the production of 2,4-dihydroxybutyric acid comprising:

- (a) providing 2,4-dihydroxybutyryl-CoA obtained by the method of claim 11; and
- (b) converting 2,4-dihydroxybutyryl-CoA to 2,4-dihydroxybutyric acid by contacting 2,4-dihydroxybutyryl-CoA with any one of (i) and (iii):
 - (i) a thioesterase or an acyl-CoA transferase catalyzing the conversion of 2,4-dihydroxybutyryl-CoA to 2,4-dihydroxybutyric acid;
 - (ii) a phosphotransacylase catalyzing the conversion of 2,4-dihydroxybutyryl-CoA to 2,4-dihydroxybutyryl-phosphate and a carboxylate kinase catalyzing the conversion of 2,4-dihydroxybutyryl-phosphate to 2,4-dihydroxybutyric acid; or
 - (iii) an acyl-CoA reductase catalyzing the conversion of 2,4-dihydroxybutyryl-CoA to 2,4-dihydroxybutanal and an aldehyde dehydrogenase catalyzing the conversion of 4-(methylthio)-2-hydroxy-butanal to 2,4-dihydroxybutyric acid.

Claim 13: A method for the production of 2-hydroxybutyrolactone comprising:

- (a) providing 2,4-dihydroxybutyryl-CoA obtained by the method of claim 11; and
- (b) lactonization of 2,4-dihydroxybutyryl-CoA to hydroxybutyrolactone spontaneously; or
- (c) providing 2,4-dihydroxybutyric acid obtained by the method of claim 12; and
- (d) adjusting pH to lower than 6 driving the lactonization of 2,4-dihydroxybutyric acid to 2-hydroxybutyrolactone;

Claim 14: A method for the production of 2-ketobutyrolactone comprising:

- (a) providing hydroxybutyrolactone obtained by the method of claim 13; and
- (b) converting hydroxybutyrolactone to 2-ketobutyrolactone by contacting hydroxybutyrolactone with a 2-hydroxy acid dehydrogenase;

Claim 15: A method for the production of 2-hydroxy-4-(methylthio)butyric acid (desmeninol) comprising:

- (a) providing hydroxybutyrolactone obtained by the method of claim 13; and
- (b) converting hydroxybutyrolactone to desmeninol by condensation of hydroxybutyrolactone with methanethiol or sodium or potassium salt thereof; or

Claim 16: A method for the production of 2-keto-4-(methylthio)butyric acid comprising:

- (a) providing 2-hydroxy-4-(methylthio)butyric acid (desmeninol) obtained by the method of claim 15; and
- (b) converting 2-hydroxy-4-(methylthio)butyric acid to 2-keto-4-(methylthio)butyric acid by contacting 2-hydroxy-4-(methylthio)butyric acid with a 2-hydroxy acid dehydrogenase; or
- (c) providing 2-ketobutyrolactone obtained by the method of claim 11; and
- (d) converting 2-ketobutyrolactone to 2-keto-4-(methylthio)butyric acid by condensation of 2-ketobutyrolactone with methanethiol or sodium or potassium salt thereof;

Claim 17: A method for the production of L-methionine or D-methionine comprising:

- (a) providing 2-keto-4-(methylthio)butyric acid obtained by the method of claim 16; and
- (b) converting 2-keto-4-(methylthio)butyric acid to L-methionine or D-methionine by contacting 2-keto-4-(methylthio)butyric acid with an enzyme selected from an amino acid dehydrogenase or a methionine aminotransferase.