

## SUPPLEMENTAL MATERIAL

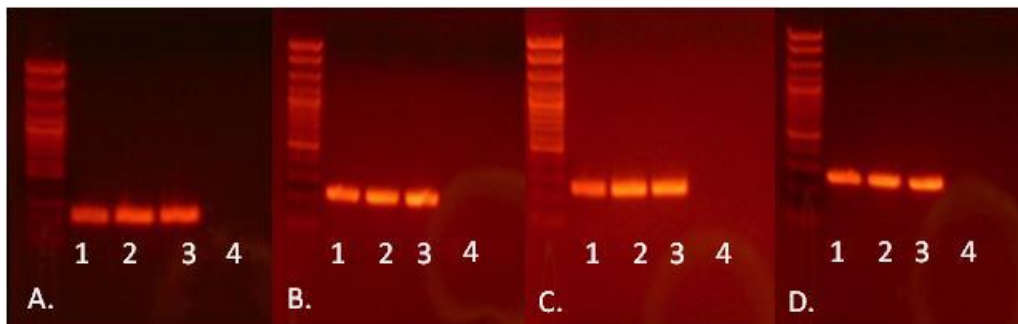


Figure S1. Amplification of 3-HP/4-HB marker genes: A) *hcd*; B) *accC*; C) *accB*; D) *pccB*. Lanes 1 & 2 = *M. yellowstonensis*; Lane 3 = Fe(III)-oxide mat environmental sample; Lane 4 = Negative *E. coli* control; Lane 5 = Reagent blank.

**Table S1.** Protein coding genes of the 3-hydroxypropionate/4-hydroxybutyrate cycle identified in the *M. yellowstonensis* strain MK1 genome and *M. yellowstonensis*-like *de novo* assemblies from the OSP Fe(III)-oxide mat metagenomes.

Enzymes of the Proposed 3-hydroxypropionate/4-hydroxybutyrate cycle	Candidate gene in <i>M. sedula</i> (9,33)	Candidate enzyme in <i>M. yellowstonensis</i> , identified through BLASTp homology to <i>M. sedula</i> - Accession (expect value, % positives)	Candidate enzyme in <i>M. yellowstonensis</i> -like HFO metagenome sequences, identified through BLASTp homology to <i>M. sedula</i> - IMG Gene ID <sup>a</sup> (expect value, % positives)
acetyl-CoA carboxylase	msed_0147/msed_0148/msed_1375	EHP69328.1 (0.0, 93%) / EHP69329.1 (4e-100, 94%) / EHP68101.1 (0.0, 97%)	152750 (0.0, 94%) / 152760 (2e-99, 94%) / 60850 (0.0, 97%)
malonyl-CoA reductase (NADPH)	msed_0709	EHP70731.1 (0.0, 95%)	143020 (1e-138, 97%)
malonate semialdehyde reductase (NADPH)	msed_1993	EHP68707.1 (2e-173, 87%)	79160 (6e-166, 88%)
3-hydroxypropionyl-CoA synthetase (AMP-forming)	msed_1456	EHP68110.1 (0.0, 95%)	60890 (0.0, 96%)
3-hydroxypropionyl-CoA dehydratase	msed_2001	EHP68697.1 (6e-144, 89%)	78860 (1e-121, 89%)
acryloyl-CoA reductase (NADPH)	msed_1426	EHP70486.1 (0.0, 90%)	183700 (4e-180, 90%)
propionyl-CoA carboxylase	msed_0147/msed_0148/msed_1375	EHP69328.1 (0.0, 93%) / EHP69329.1 (4e-100, 94%) / EHP68101.1 (0.0, 97%)	152750 (0.0, 94%) / 152760 (2e-99, 94%) / 60850 (0.0, 97%)
methylmalonyl-CoA epimerase	msed_0639	EHP70648.1 (7e-80, 91%)	71510 (3e-79, 91%)
methylmalonyl-CoA mutase	msed_0638/msed_2055	EHP70647.1 (0.0, 95%) / EHP68637.1 (5e-80, 94%)	154270 (2e-175, 93%) / 28150 (6e-70, 94%)
succinyl-coA reductase (NADPH)	msed_0709	EHP70731.1 (0.0, 95%)	143020 (1e-138, 97%)
succinate semialdehyde reductase (NADPH)	msed_1424	EHP70477.1 (0.0, 93%)	57730 (0.0, 93%)
4-hydroxybutyryl-CoA ligase (AMP-forming)	msed_0394, msed_0406	EHP71147.1 (0.0, 88%), EHP69042.1 (0.0, 87%)	39960 (0.0, 89%), 155470 (0.0, 88%)
4-hydroxybutyryl-CoA dehydratase, Type I	msed_1321	EHP69763.1 (0.0, 97%)	44600 (0.0, 98%)
crotonyl-CoA hydratase	msed_0399	EHP69048.1 (0.0, 81%)	155420 (0.0, 82%)
3-hydroxybutyryl-CoA dehydrogenase	msed_0399	EHP69048.1 (0.0, 81%)	155420 (0.0, 82%)
acetoacetyl-CoA-B-ketothiolase	msed_0656	EHP70668.1 (0.0, 88%)	71440 (0.0, 88%)

"/" separates subunits of enzyme complex, "," separates proposed candidate genes

<sup>a</sup> Actual IMG Gene ID is formatted as "YNPsite14\_CeleraDRAFT\_" followed by the ID-number reported

**Table S2.** Major ion constituents used in synthetic media for growth experiments using *M. yellowstonensis* strain MK1.

<b><u>Cations</u></b>	<b><u>uM</u></b>	<b><u>Anions</u></b>	<b><u>uM</u></b>
Na	15800	NO <sub>3</sub>	400
K	1547	SO <sub>4</sub>	1212
Al	114	Cl	10642
Mg	12	PO <sub>4</sub>	100
NH <sub>4</sub>	600	F	147
Ca	250	B	717
		CO <sub>3</sub>	5000
C:N:P	50:10:01		