

## SUPPLEMENTAL MATERIAL

**TABLE S1** Primers designed to amplify the targeted gene transcripts in *M. conradii*.

Gene name	Locus tag	Primer Name	Primer sequence(5'-3')
16S rRNA	Mtc_r6	H16S-F	TACCGTGAGGCGTCTGTTAAG
		H16S-R	GCCAGCAGTCTCCACAGTGTA
<i>echE</i>	Mtc_0802	echE-F	GGATAGAGCCTCTCCGCTTGAA
		echE-R	TAGCACACACCCTCTCCGAAAG
<i>frhA1</i>	Mtc_1001	frhA-1F	AAGGCTACCGAAGGCTTCCATT
		frhA-1R	CCACCTATCCTCCACGACCTTG
<i>frhA2</i>	Mtc_2127	frhA-2F	CACCGTCCGCATCCAGAAGA
		frhA-2R	CATGCCGCCGATCCTTATGTTC
<i>hdrB1</i>	Mtc_1470	hdrB-1F	GTGAGAGCCGTGACCCATCAT
		hdrB-1R	GTCCAGCACGCTACACCCTAA
<i>hdrB2</i>	Mtc_2474	hdrB-2F	CGGAGGAGTGCGAGGCTATAAG
		hdrB-2R	CTGGAGGTGACAGAACGGACAA
<i>hdrB3</i>	Mtc_0481	hdrB-3F	TACGAAGTCCACCTGCTGCC
		hdrB-3R	CGCCTCCTCTGCCTGAAGTAA
<i>mvhA1</i>	Mtc_0109	mvhA-1F	GGCATGGCGTTCTCGCTTAC
		mvhA-1R	CGTTCACCAGGTCTGCGTACT
<i>mvhA2</i>	Mtc_2479	mvhA-2F	CGTGTATCCGACTCCTACCTT
		mvhA-2R	ACTCCTGAAGCTCGGCGTTAG
<i>vhtG</i>	Mtc_0470	vhtG-F	CCCTACCAGGCGATGGAATGAT
		vhtG-R	TGCTCTCCACCTTGCTGAAGAA
<i>mcrA</i>	Mtc_0908	mcrA-F	ATCTGGCTCGGCTCCTACATG
		mcrA-R	GGCTTGGTCGGCATCTTGTAC
<i>mtrH</i>	Mtc_0943	mtrH-F	GCAAAGAGGGCGGACGAGAT
		mtrH-R	CGATGGCAGCGGTGAACTTC
<i>mtd</i>	Mtc_1592	Mtd-F	CCGAACCTGCCGCAGATAGT
		Mtd-R	ACGTCGATGTCGCCAACCTT
<i>mer</i>	Mtc_0135	mer-F	TCGACCAGGACGCCAAGAAG
		mer-R	CCGCCTTAATCGTGCCAACC
<i>fwdB</i>	Mtc_2470	fwdB-F	GGAGGATGGCACCCTGAGTT
		fwdB-R	TCGCACGACGTTGAAGACCA
<i>fmdB</i>	Mtc_0163	fmdB-F	GGTCAAGCAGGAGCAGGAAGT
		fmdB-R	CCATCAGGCAAGCGTTCTTCG
<i>fdhA</i>	Mtc_2125	fdhA-F	AATATGCCTGCTCGCCCAGAA
		fdhA-R	TCGGAGCCTCTCGTAGGTCAT
<i>porA1</i>	Mtc_0842	porA-1F	TACCGGCCATTCCTCCGCTGA
		porA-1R	CGCGAAGCCCTTCCTTGCCA
<i>porA2</i>	Mtc_1765	porA-2F	GCCTGCCATGGTCTGCCTGG
		porA-2R	CCCGCTTCATGGCGTCGTGT
<i>acs1</i>	Mtc_1904	acs-1F	ATGGGCAAAGGCCGTGTGGG
		acs-1R	CTCACCCGCCACCACGCAAT
<i>acs2</i>	Mtc_2228	acs-2F	TGGAGCCGGGAGACCTGAGC
		acs-2R	CCCATCCCGGGTCAGCCGTA
<i>ppa</i>	Mtc_1504	ppa-F	GGGGCAGCCGGTAGACCTGA
		ppa-R	ATGGCTGCAAGGGCCGTCAC

**TABLE S2** Genes encoding enzymes for hydrogenotrophic methanogenesis pathway in *M. conradii*.

<b>Enzyme</b>	<b>gene or gene cluster</b>	<b>Locus tag</b>
Energy-converting hydrogenase	<i>echABCDEFG</i>	Mtc_0806-0801
Methanophenazine-reducing [NiFe] hydrogenase	<i>vhtGCA</i>	Mtc_0470-0472
	<i>vhtD</i>	Mtc_2406
	<i>frhA2DG2B2</i>	Mtc_2127-2130
Coenzyme F <sub>420</sub> -reducing hydrogenase	<i>frhB1G1A1</i>	Mtc_0999-1001
	<i>frhB3</i>	Mtc_1195
	<i>mvhG2A2</i>	Mtc_2478-2479
Coenzyme F <sub>420</sub> -non-reducing hydrogenase (methylviologen-reducing hydrogenase)	<i>mvhA1G1</i>	Mtc_0109&0111
	<i>mvhD2</i>	Mtc_2472
CoB-CoM heterodisulfide reductase	<i>hdrA2B2C2</i>	Mtc_2473-2475
	<i>hdrC1 hdrA1/mvhD1 hdrB1</i>	Mtc_1469-1471
	<i>hdrB3</i>	Mtc_0481
	<i>hdrC-like</i>	Mtc_p13
Formylmethanofuran dehydrogenase (tungsten containing)	<i>fwdCABD</i>	Mtc_2468-2471
	<i>fwdFG</i>	Mtc_2476-2477
	<i>fwdE1</i>	Mtc_0156
	<i>fwdE2</i>	Mtc_1680
	<i>fwdE3</i>	Mtc_1818
Formylmethanofuran dehydrogenase (molybdenum containing)	<i>fmdBD</i>	Mtc_0163-0164
	<i>fmdE</i>	Mtc_0160
Formylmethanofuran:H <sub>4</sub> MPT formyltransferase	<i>fr</i>	Mtc_1916
	<i>mch1</i>	Mtc_1063
Methenyl-H <sub>4</sub> MPT cyclohydrolase	<i>mch2</i>	Mtc_1978
Methylene-H <sub>4</sub> MPT dehydrogenase	<i>mtd</i>	Mtc_1592
Methylene-H <sub>4</sub> MPT reductase	<i>mer</i>	Mtc_0135
Methyl-H <sub>4</sub> MPT:coenzyme M methyltransferase	<i>mtrEDCBA1FGH</i>	Mtc_0936-0943
	<i>mtrA2</i>	Mtc_0962
putative methyltransferase mtX	<i>mtxX</i>	Mtc_0232
Methyl-coenzyme M reductase	<i>mcrBDCGA</i>	Mtc_0904-0908
Formate dehydrogenase	<i>fdhBAC</i>	Mtc_2124-2125
	<i>fdhD1&amp;D2</i>	Mtc_0165 & 0273

## SUPPLEMENTAL FIGURE LEGENDS

**FIGURE S1** Hydrogenase encoding gene clusters and methanogenesis-associated gene clusters in *M. conradii* according to the annotation of the *M. conradii* genome (1). Red coloration indicates the catalytic sites and blue coloration and blue arrows highlight the genes in a large transcription unit corresponding to the description below the genes.

A), Energy-converting hydrogenase [NiFe] [FeS] (Ech)

B), MP-reducing hydrogenase [Cytb] [NiFe][FeS] (Vht, membrane-integrated)

C), F<sub>420</sub>-reducing hydrogenase [NiFe] [FeS] (Frh)

D), F<sub>420</sub>-non-reducing hydrogenase [NiFe] [FeS] (Mvh, methyl viologen-reducing hydrogenase)

E), CHO-MFR dehydrogenase [Mo] [MPT] [FeS] (Fmd)

F), Formate dehydrogenase (Fdh)

G), CHO-MFR dehydrogenase [W] [MPT] [FeS] (Fwd)

H), CHO-MFR:H<sub>4</sub>MPT formyltransferase (Ftr) and Methenyl-H<sub>4</sub>MPT cyclohydrolase [Ca<sup>2+</sup>] (Mch)

I), Methylene-H<sub>4</sub>MPT dehydrogenase (Mtd) and Methylene-H<sub>4</sub>MPT reductase (Mer)

J), Methyl-H<sub>4</sub>MPT:CoM methyltransferase [B<sub>12</sub>(Co<sup>1+</sup>)] (Mtr)

K), Methyl-CoM reductase [F<sub>430</sub>(Ni<sup>1+</sup>)] (Mcr)

L), Heterodisulfide reductase [FeS] [Zn<sup>2+</sup>] (Hdr) and Ech-like hydrogenase

### Reference:

1, Lü, Z., and Y. H. Lu. 2012. Complete genome sequence of a thermophilic methanogen, *Methanocella conradii* HZ254, isolated from Chinese rice field soil. J. Bacteriol. **194**:2398-2399.

FIGURE S1

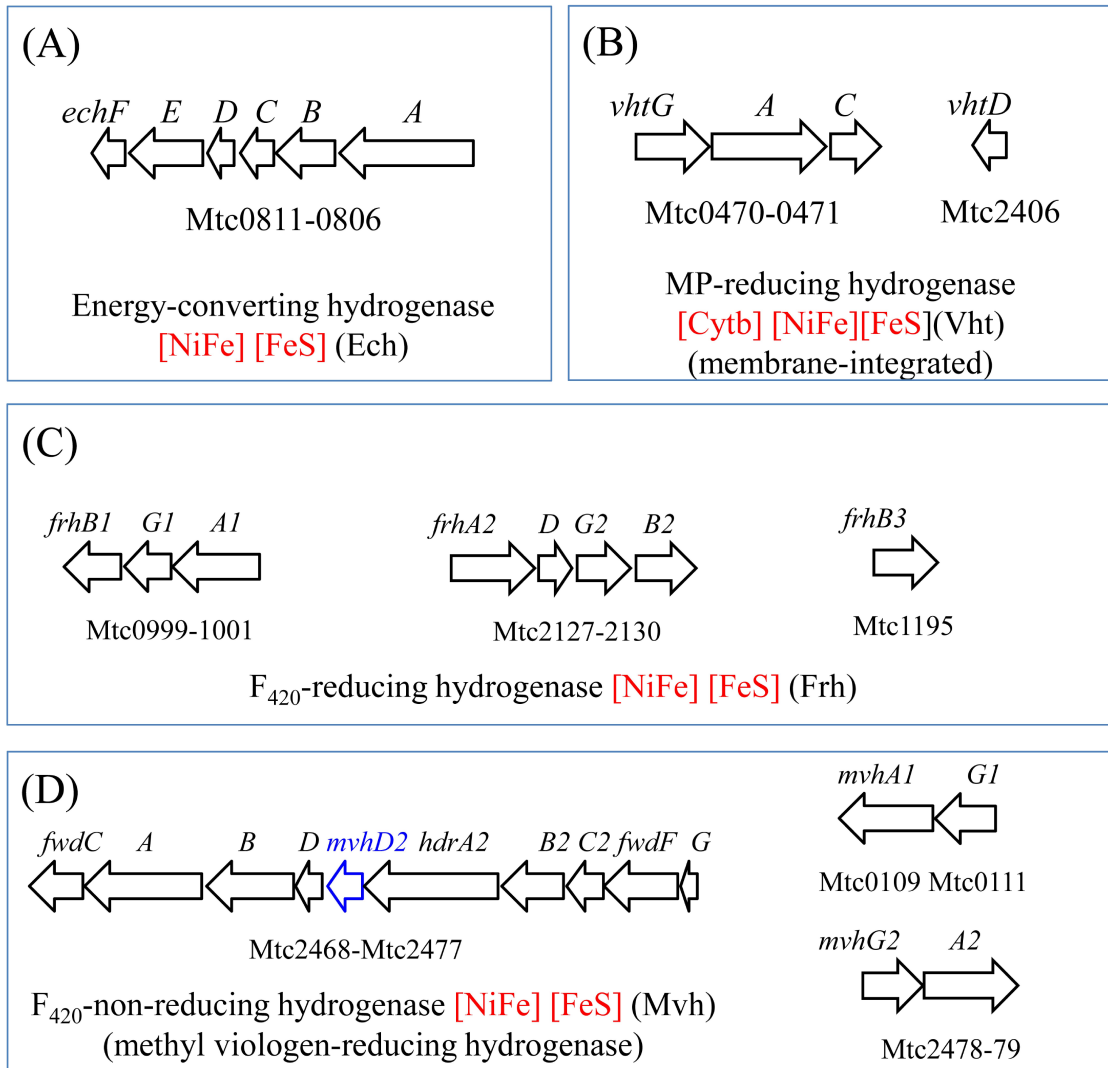


FIGURE S1 CONTINUED

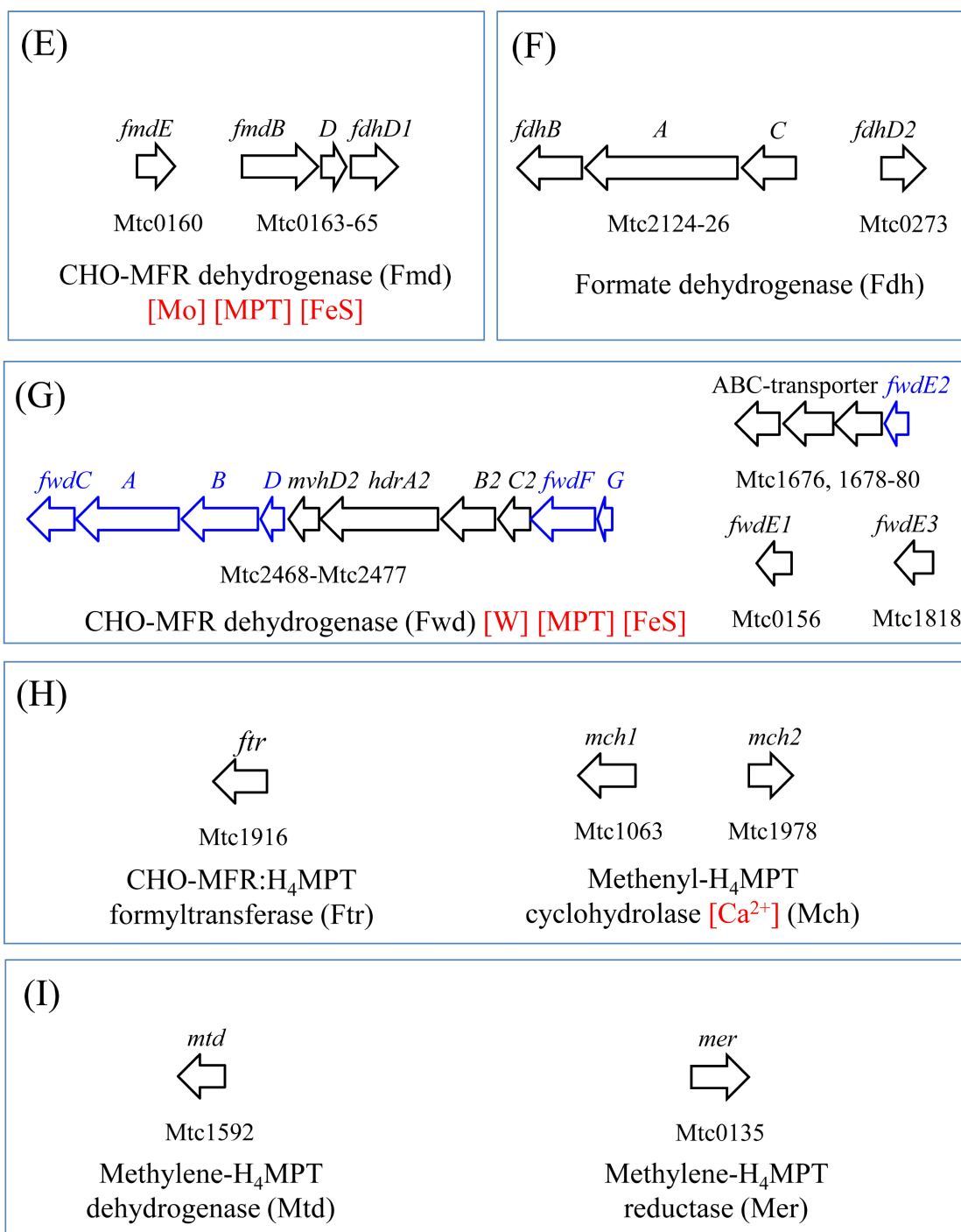


FIGURE S1 CONTINUED

