Supporting Information

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SI Text

Sequence Analysis of Fpo Subunits from *Methanosarcina* Species. The input module, FpoF, has been proposed to oxidize $F_{420}H_2$ and transfer electrons via enzyme-bound flavin adenine dinucleotide to 2 tetranuclear iron-sulfur [4Fe-4S] clusters that are bound to FpoF by cysteine-containing motifs, $C^{14}XXC^{17}XXC^{20}XXXC^{24}P$ and $C^{55}XXC^{58}XXC^{61}XXXC^{65}P$, which are conserved in all 3 *Methanosarcina* FpoF subunits (Fig. S3) (1). These subunits share 86% to 89% amino acid identity (Table S1). Interestingly, the genomic location of *fpoF* is conserved in all 3 species, such that it is located between genes encoding methylenetetrahydrosarcinapterin reductase (*mer*) and a putative cytidylyltransferase (Fig. S1) (2). Although it is unknown if the 3 genes constitute an operon, the *mer-fpoF* intergenic region in all 3 *Methanosarcina* species includes multiple polyT tracts, which are known to signal transcription termination in Archaea (Fig. S4) (3).

Electrons from FpoF probably are channeled through the membrane-associated module of Fpo, Fpo BCDI, via 3 [4Fe-4S] clusters in FpoI and FpoB. Of these [4Fe-4S] clusters, 2 are bound to FpoI by motifs $C^{44}XXC^{47}XXC^{50}XXXC^{54}P$ and $C^{84}XXC^{87}XXC^{90}XXXC^{94}P$ (Fig. S3) and are assumed to be analogous to the N6a and N6b clusters of Nuo of *Escherichia coli*. On the other hand, the third [4Fe-4S] cluster has been compared with the N2 cluster of Nuo of *E. coli* and probably binds FpoB via the motif $C^{61}C^{62}XXEX_{60}C^{126}X_{29}C^{156}P$. All 3 [4Fe-4S] cluster-binding motifs are well conserved in the 3 *Methanosarcina* FpoI and FpoB subunits (1, 4, 5). These subunits share a high degree of amino acid sequence identity (>83%) (Table S1). Although the amphipathic subunits FpoC and FpoD do not have well-defined functions, FpoD has been implicated in binding methanophenazine aromatic rings via the conserved Glycine^{367} residue. FpoD also contains 2 conserved cysteines, C^{70} and C^{368}, which are reminiscent of its ancestral protein, the large subunit of [NiFe] hydrogenases that harbors these cysteines as Ni-binding ligands (Fig. S3) (4). Both the FpoC and FpoD subunits from the 3 *Methanosarcina* species show significant amino acid sequence identity of 80% to 90% (Table S1). Another Fpo subunit that has eluded characterization is the hydrophilic FpoO subunit. In *M. mazei*, FpoO contains a [2Fe-2S] binding motif, $SC^{58}RXGXC^{63}SXC^{66}XXKX_{24}C^{94}$ (Fig. S3) (1). The 4 cysteines of the motif that coordinate the iron-sulfur center are conserved in the other 2 *Methanosarcina* species. However, *M. acetivorans* is missing the Serine^{64} residue, and *M. barkeri* lacks the Glycine^{61} and Serine^{64} residues. *M. acetivorans* has an additional copy of *fpoO* (*fpoO2*) that is approximately 3 kb downstream of the first copy in the *fpo* operon (*fpoO1*) (Fig. S1) (2). Both FpoO subunits show 79% amino acid identity, but FpoO2 lacks 1 of the cysteines (C^{94}) that coordinates the iron-sulfur cluster. The FpoO subunits from *M. mazei* and *M. acetivorans* show 80% amino acid identity, but *M. barkeri* FpoO shows only *ca.* 60% identity to the other 2 FpoO subunits.

Electrons from FpoF through FpoI and then FpoB are thought to be transferred to the membrane-integral module of Fpo, FpoAHJKLMN, which uses them to reduce methanophenazine by an as yet unknown mechanism (5). All hydrophobic Fpo subunits possess 77% to 99% amino acid sequence identity among the 3 *Methanosarcina* species (Table S1). Also, in all of the species, FpoJ is encoded by 2 contiguous *fpoJ* genes, such that the first gene encodes a protein that is homologous to the amino terminus of NuoJ, and the second encodes a protein that is homologous to the carboxyl terminus of NuoJ (2). Interestingly, *M. acetivorans* Fpo has been proposed to contain a small additional subunit, FpoP, when grown on CO as the growth substrate (6). However, there is no experimental evidence that FpoP protein is produced, nor is the gene conserved in the other 2 *Methanosarcina* species (2). Thus, it still is unknown if FpoP has a role in methanogenesis from any substrate. In conclusion, the *in silico* analysis of Fpo predicted protein sequences from the 3 *Methanosarcina* species shows conservation of all important catalytic and structural residues in the proteins, suggesting that they may be functional.

Materials and Methods

Construction of *M. barkeri* Fusaro Deletion Mutants. The markerless genetic exchange method (7) was used to delete the *fpoA-O* operon (*fpo*), *fpoF*, and *freAEGB* operon (*fre*) in the Δ*hpt* (WWM85 or WWM86) (8) background of *M. barkeri* Fusaro (Table S2). DNA sequences immediately flanking the deleted genes were left intact to exclude loss of regulatory elements needed for expression of adjacent genes. The plasmids pDK4, pDK13, and pGK6 were used to delete *fpoA-O* in WWM86, *fpoF* in WWM85, and *fre* in WWM85, respectively, on methanol plus H_2/CO_2 as the growth substrate. The *frhADGB* operon (*frh*) was deleted in the Δ*hpt* (WWM86), Δ*fpo* (WWM71), and Δ*fpoF* (WWM123) backgrounds of *M. barkeri* Fusaro by the homologous recombination-mediated gene replacement method (9). In this method, the ApaI/NotI-cut 5.5-kb region of pAMG81 was transformed into Δ*hpt*, Δ*fpo*, and Δ*fpoF* mutants, and the transformants were selected on methanol plus H_2/CO_2 and puromycin to obtain Δ*frh* (WWM122), Δ*fpo*/Δ*frh* (WWM108), and Δ*fpoF*/Δ*frh* (WWM145) mutants, respectively. All of the mutants were confirmed by DNA hybridization. All genetic manipulations were carried out using methanol plus H_2/CO_2 as the growth substrate because the $F_{420}H_2$:heterodisulfide oxidoreductase system was not expected to be required for growth via the methyl-respiration pathway.

Determination of Growth Characteristics. For growth rate determination, cultures were adapted to all substrates for at least 15 generations or, in the absence of growth on a particular substrate, were grown on methanol plus H_2/CO_2 to mid-log phase (OD_{600}, *ca.* 0.5). An approximately 3% inoculum of the culture then was transferred to fresh medium in quadruplets and incubated at 37 °C. Growth substrates provided were methanol (125 mM) or sodium acetate (120 mM) under a headspace of either N_2/CO_2 (80%/20%) at 50 kPa over ambient pressure or H_2/CO_2 (80%/20%) at 300 kPa over ambient pressure. Puromycin (CalBioChem) was added at 2 μg/mL for selection of the puromycin transacetylase (*pac*) gene (7, 10). 8-aza-2,6-diaminopurine (8-ADP) (Sigma) was added at 20 μg/mL for selection against the presence of *hpt* (7). Lag phase was defined as the time required to achieve half-maximal OD_{600}.

Cell Suspension Experiments. Cells grown on methanol plus H_2/CO_2 were collected in late-exponential phase (OD_{600} = 0.6–0.7) by centrifugation at 5000 × *g* for 15 min at 4 °C. The cells were washed once with anaerobic high-salt Pipes buffer, 50 mM Pipes (pH 6.8), 400 mM NaCl, 13 mM KCl, 54 mM $MgCl_2$, 2 mM $CaCl_2$, 2.8 mM cysteine, 0.4 mM Na_2S, and were resuspended in the same buffer to a final concentration of 10^9 cells/mL. Assay mixtures contained 2 mL of the suspension and were incubated under strictly anaerobic conditions in 25-mL Balch tubes sealed with butyl rubber stoppers. Puromycin (20 μg/mL) or Mupirocin (pseudomonic acid, 105 μg/mL, Sigma-Aldrich) (11) was added

to prevent protein synthesis and, as indicated, the assay mixture contained 250 mM methanol and a headspace of N_2, H_2, or H_2/CO_2 (80%/20%) at 250 kPa over the ambient pressure. Cells were held on ice until use, and assays were started by transferring to 37 °C. For rate determination, gas-phase samples were withdrawn at various time points and assayed for CH_4 by gas chromatography at 225 °C in a Hewlett-Packard gas chromatograph (5890 Series II) equipped with a flame ionization detector. The column was of stainless steel filled with 80/120 Carbopack B/3% SP-1500 (Supelco) with helium as the carrier gas. For total CH_4 and CO_2 production, assays were incubated at 37 °C for 36 h; then gas phase samples were withdrawn. These samples were analyzed by gas chromatography at 225 °C in a Hewlett-Packard gas chromatograph (5890 Series II) equipped with a thermal conductivity detector. A stainless steel 60/80 Carboxen-1000 column (Supelco) with helium as the carrier gas was used. Total cell protein was determined using the Bradford method (12) after 1 mL of the cells was lysed by resuspending the pellet in double-distilled H_2O with 1 μg/mL RNase and DNase.

RNA Isolation and Quantitative RT-PCR. Cultures grown to mid-exponential phase (OD_{600} *ca.* 0.28 for Δ*frh* and *ca.* 0.4 for other strains) on methanol were lysed using 3 volumes of TRIzol LS Reagent (Invitrogen), followed by phase separation using chloroform according to the manufacturer's instructions. Subsequently, RNA was precipitated using 1 volume of 70% ethanol and purified using RNeasy mini spin columns (Qiagen) according to the manufacturer's protocol. Contaminating DNA was removed by treatment with TURBO DNA-*free* DNase (Ambion). The concentration and purity of RNA were evaluated using a NanoDrop spectrophotometer (NanoDrop).

All reactions (20 μL) contained 1X SYBR Green Reaction Mix with ROX, 0.4 μL SuperScript III RT/Platinum Taq mix, 100 ng RNA, and 10 pmol each of primers. Synthesis of cDNA from RNA and subsequent amplification were performed as follows: 50 °C for 5 min and 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. We ran 2 negative controls, 1 without reverse transcriptase and another without template RNA, to check for DNA or RNA contamination.

Standard curves relating cycle threshold values versus log amount of RNA were constructed for *fpo* and *rpoA1* using RNA isolated from methanol-grown WWM85 (Table S2). The amount of *fpo* RNA in each sample was calculated using linear regression of the standard curve and averaged across triplicates of 3 biological samples for each culture. The average *fpo* amount was normalized to the average *rpoA1* amount to obtain the relative *fpo* amount. This value was divided by the calibrator value (WWM85) to obtain *fpo* fold regulation from wild type.

1. Baumer S, et al. (2000) The $F_{420}H_2$ dehydrogenase from *Methanosarcina mazei* is a redox-driven proton pump closely related to NADH dehydrogenases. *J Biol Chem* 275:17968–17973.
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14. Welander PV, Metcalf WW (2008) Mutagenesis of the C1 oxidation pathway in *Methanosarcina barkeri*: New insights into the Mtr/Mer bypass pathway. *J Bacteriol* 190:1928–1936.

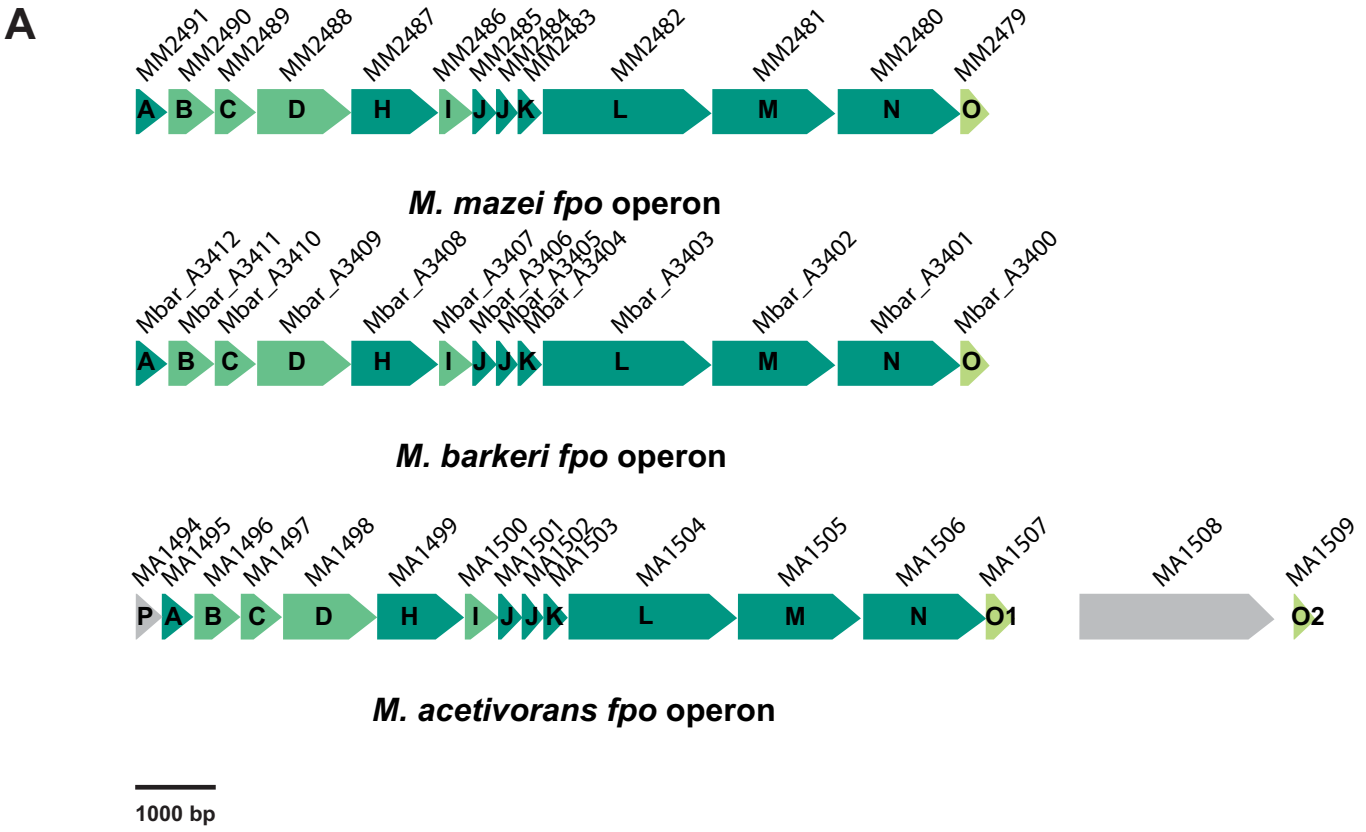
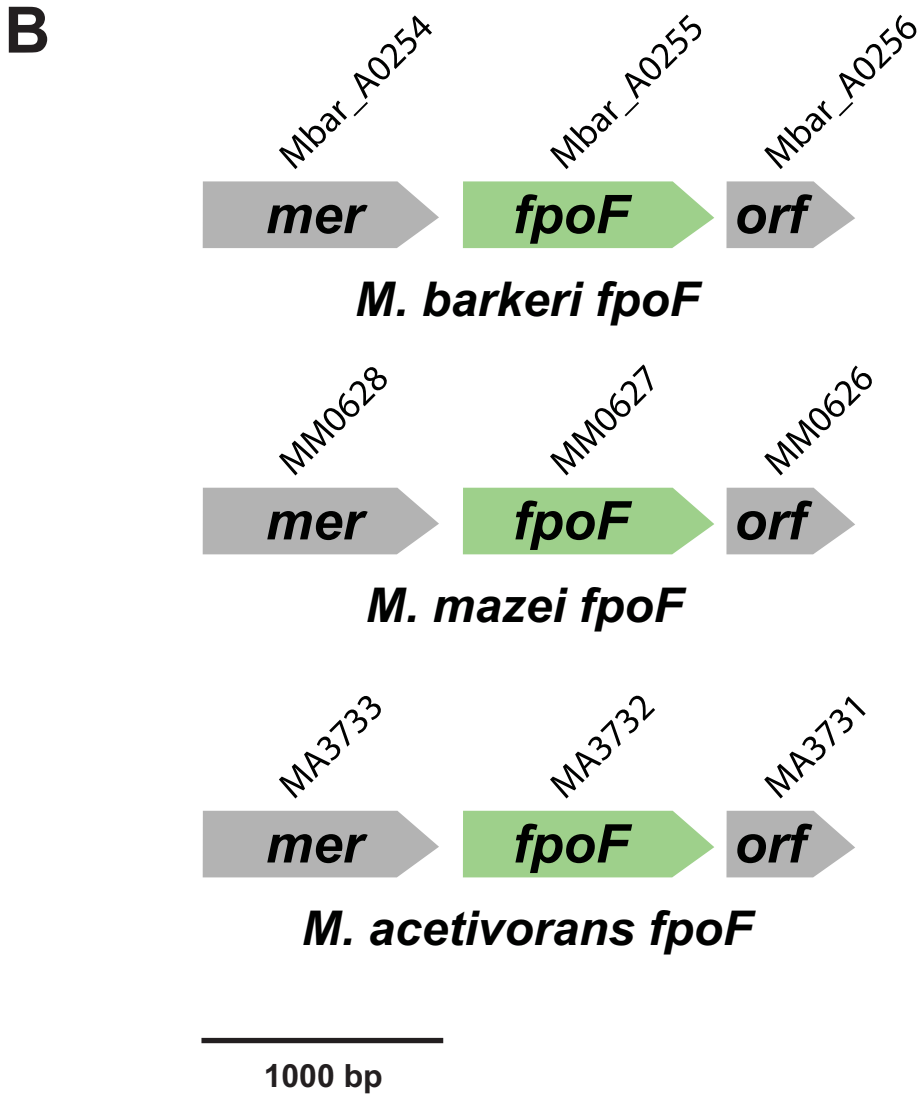


Fig. S1. Genomic organization of operons encoding F_{420}-phenazine oxidoreductase (Fpo), F_{420}-reducing hydrogenase (Frh), and heterodisulfide reductase (Hdr) in *Methanosarcina* species. (*A*) The 13-gene operon (*fpoABCDHIJJKLMNO*) that encodes the hydrophilic (FpoO), membrane-associated (FpoBCDI), and membrane-integral modules (FpoAHIJKLMN) of Fpo in the 3 species. *M. acetivorans* harbors 2 copies of *fpoO*, *fpoO1* and *fpoO2*, and *fpoP* that are not present in the other 2 *Methanosarcina* species. (*B*) The gene (*fpoF*) that encodes the input module of Fpo (FpoF). In all 3 species, *fpoF* is located between genes encoding methylenetetrahydrosarcinapterin reductase (*mer*) and a putative cytidylyltransferase (2). These genes may constitute an operon. (*C*) The F_{420}-reducing hydrogenase operons (*frhADGB*) in the 3 species. *M. barkeri* has 2 of these operons, *frhADGB* and *freAEGB*. The *freAEGB* operon lacks gene *D* that encodes a putative hydrogenase maturation protein assumed to be essential for posttranslational modification of the *cis*-encoded hydrogenase. (*D*) The operons encoding heterodisulfide reductase (HdrED) in the 3 species. Other ORFs are shown as gray arrows.



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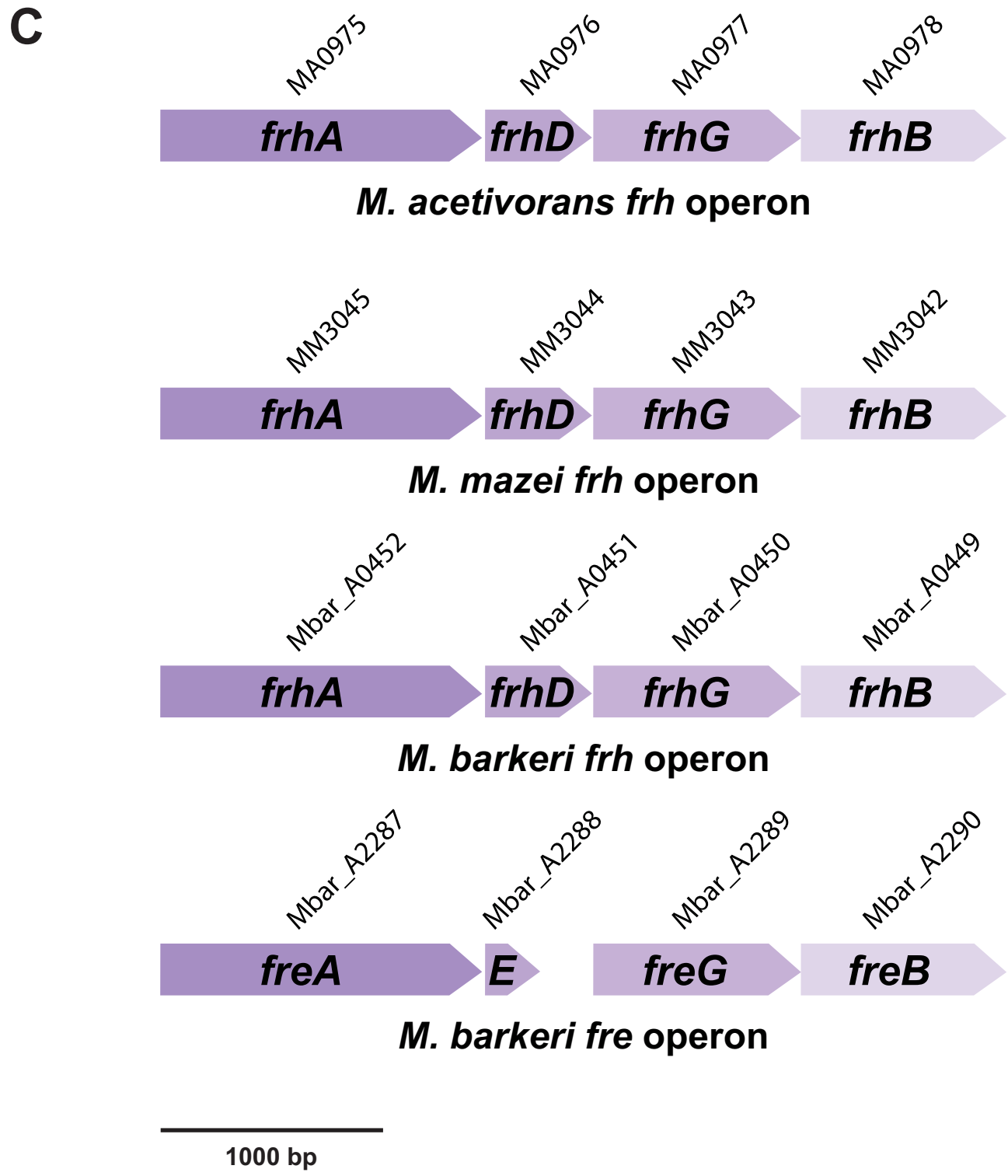

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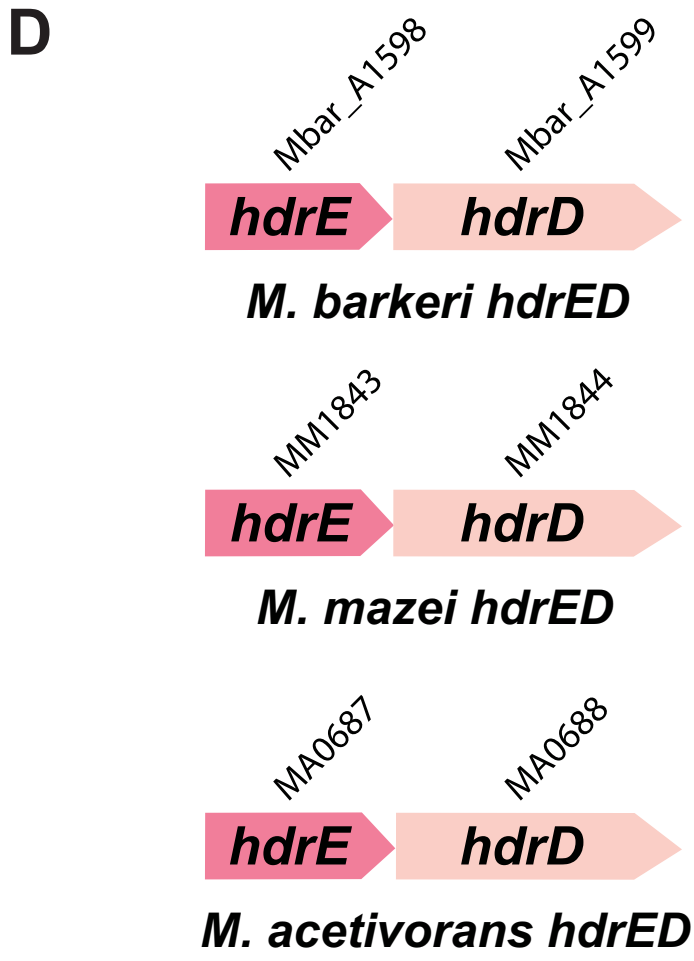

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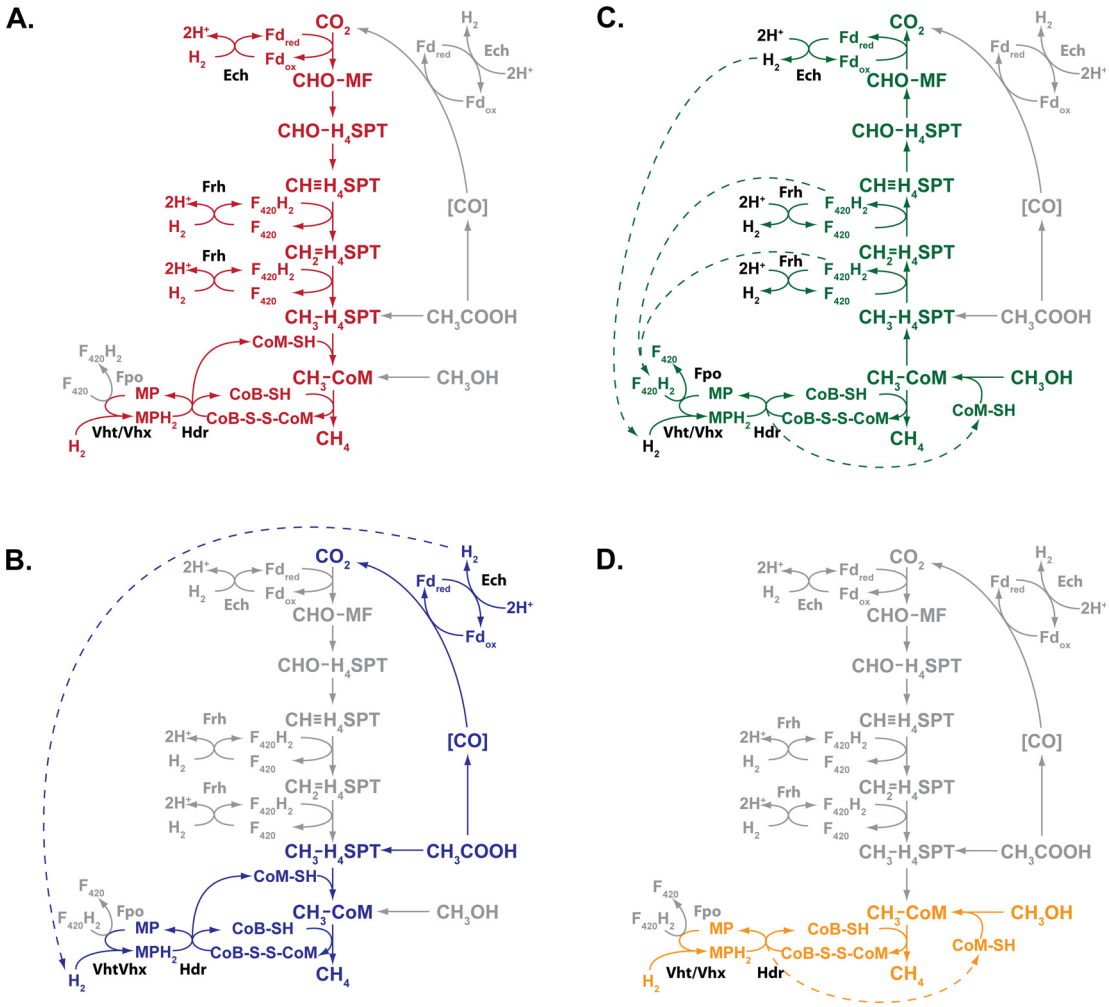

Fig. S2. Four overlapping methanogenic pathways found in *M. barkeri* (adapted from ref. 13). Each pathway shares a common step in the reduction of methyl-CoM to methane; however, they differ in the route used to form methyl-CoM and in the source of electrons used for its reduction to methane. (*A*) Many methanogens reduce CO_2 to methane using electrons derived from the oxidation of H_2 (hydrogenotrophic pathway). (*B*) Alternatively, acetate can be split into a methyl group and an enzyme-bound carbonyl moiety. The latter is oxidized to CO_2 to provide electrons required for the reduction of methyl group to methane (aceticlastic pathway). (*C*) C1 compounds such as methanol or methylamines also can be disproportionated to CO_2 and methane. In this pathway, 1 molecule of the C1 compound is oxidized to provide electrons for reduction of 3 additional molecules to methane (methylotrophic pathway). (*D*) C1 compounds can be reduced using electrons derived from H_2 oxidation (methyl respiration pathway). Steps not required by each pathway are shown in gray. The steps catalyzed by Fpo, Frh, Vht/Vhx, Ech, and Hdr proteins are indicated. Note that Fpo is predicted to be required in the methylotrophic pathway and Frh in the hydrogenotrophic pathway. Abbreviations: CH-- H_4SPT, methylene-tetrahydrosarcinapterin; CH≡H_4SPT, methenyl-tetrahydrosarcinapterin; CH_3- H_4SPT, methyl-tetrahydrosarcinapterin; CH_3-CoM, methyl-coenzyme M; CHO-H_4SPT, formyl-tetrahydrosarcinapterin; CHO-MF, formyl-methanofuran; CoB-SH, coenzyme B; CoM-SH, coenzyme M; CoM-S-S-CoB, mixed disulfide of CoM-SH and CoB-SH; $F_{420}/F_{420}H_2$, oxidized and reduced Factor 420; Fd_{ox}/Fd_{red}, oxidized and reduced ferredoxin; Fpo, $F_{420}H_2$:phenazine oxidoreductase; Frh, F_{420}-reducing hydrogenase; Hdr, heterodisulfide reductase; MP/MPH_2, oxidized and reduced methanophenazine; Vht/Vhx, methanophenazine-dependent hydrogenase. Ech, Fd-dependent hydrogenase.

1. FpoF

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Mm FpoF         MPPKIAEVIQHDVCAACGACEAVCPIGAVTVKKAAEIRDPNDLSLYEKGAAFQVCEGCLT 60
Ma FpoF         MPPKIAEVIDYDVCAACGACEAVCPIGAVTVKKAAGIRDPNDLSLYEKGGGYQVCEGCLT 60
Mb FpoF         MPPKIAEVIEHDVCAACGACEAVCPIGAVTVRKAAEIRDPNDPNLYQKGAGYLVCEGCLT 60

Mm FpoF         CSRICPVVDGFIENELLNVRKFFGAKSKDNAGSQDGGVTSGILKALFNKGEIDCAVGITR 120
Ma FpoF         CSRVCPVVDGFIQDELTNVRKFFGARSKDNVGSQDGGVASGILKSLFKQGKIDCAVGITR 120
Mb FpoF         CSRICPVVDGFIENELANVRKFFAARSKENAGSQDGGVTSGILKSLFKQGKIDCAVGITR 120

Mm FpoF         NENWEPEVVLLTSAEDVERTRGTKYTSDPVVAALREAFEKYDRIAVVGVPCQAHAARLIR 180
Ma FpoF         NEKWEPEVVLLTSAEDVERTRGTKYTSDPVLAALREAFEKYDRIAVIGVPCQAHAAHLIR 180
Mb FpoF         DEKWESKVVLLTSAEDVEKVRGTKYTSDPVVAALREAFEKYDRIAVVGVPCQAHSARLIR 180

Mm FpoF         ENVNEKIVLIIGLLCMESFHHDVMLDKIIPEIMKVNVRDIVKMEFTKGKFWVYTKDGEVH 240
Ma FpoF         ENVNEKIVLIIGLLCMESFHHDVMLEKIIPEILKVKLEDIRKMEFTKGKFWVYTKDGEVH 240
Mb FpoF         ENVSEKIVLIIGLLCMESFHHDVMLDKIIPEIMKVKIEDVRKMEFTKGKFWVYTSDGEVH 240

Mm FpoF         SVPIKDIAKYARNPCHHCCDYTSVFADISVGSVGAPDGWNSVFIRTEIGEKYFDMVRDEM 300
Ma FpoF         SVPIKDVAKFARNPCHHCCDYTSVFADISVGSVGAPDGWNSVFIRTEIGEKYFDMVREDM 300
Mb FpoF         SVPIKDVAKYARNPCHHCCDYTSVFADISVGSVGAPDGWNSVFIRTDAGEEYFEMVREEM 300

Mm FpoF         EIMEDPKPGLELVGKLIEMKRKGNAEHFQEVCKEFSFETGIRSETV 346
Ma FpoF         EIMEDPKPGLELVEKLIGMKRKGNAEHFLEVCEKFSFETGIRNETI 346
Mb FpoF         EIMEDPKPGLELVKKLIDMKRKNNAEHFKEVCKEFSFETGIRDETV 346
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2. FpoA

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Ma FpoA         -----MSGIIDSYIPVAIFLAVGLIMPPMTMFMVKQLSPRSKAASKYTTYESGSIPTGTA 55
Mm FpoA         MIGDTMSGIIDSYIPVAIFLAVGLIMPPMTMFMVKQLSPRSKAASKYTTYESGSVPTGTA 60
Mb FpoA         -----MSEIIDSYIPVAIFLVVALIMPPMTMFMVKQLSPRSKAAGKYTTYESGSVPTGTA 55

Ma FpoA         RIQFNVEYYLYAIAFVLFDIEVLFLYPWATVYKGHGITSIAVVEMFVFIFILLFGYVYLW 115
Mm FpoA         RIQFNVEYYLYAIAFVLFDIEVLFLYPWATVYKGHGITSIAVVEMFVFIFILLFGYVYLW 120
Mb FpoA         RIQFNVEYYLYAIAFVLFDIEVLFLYPWVTVYKGHGITSLAVVEMFAFIFILLFGYIYLW 115

Ma FpoA         KKEALTWVK 124
Mm FpoA         KKEALTWVK 129
Mb FpoA         KKGALTWVK 124
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3. FpoB

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Ma FpoB         MGEVKETKTNNTTGTPEEEIPGVITTTTNAISDFLKKTKAQDLINWGRKNSLWFMTQPMG 60
Mm FpoB         MGEVKETKTNNSKENPEEEVPGVITTTTSAIHNFLKKTKAQDIINWGRKNSLWFMTQPMG 60
Mb FpoB         MGEVKEKKTSKPYETSEEEIPGVITTTSNAISEFLKKTKVQDIINWGRKNSLWFMTQPMG 60
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Fig. S3. F_{420}:phenazine oxidoreductase (Fpo) subunit alignments. Inferred protein sequences were aligned using ClustalW for each Fpo subunit from *M. barkeri* (Mb), *M. mazei* (Mm), and *M. acetivorans* (Ma). Green bases denote conserved amino acid residues. Red bases highlighted in yellow are proposed to coordinate iron-sulfur centers or bind the substrate methanophenazine. Refer to text for Fpo sequence analysis.

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Ma FpoB         CCGVEMIATGCAHYDTDRFGIIPRNSPRHADVMIISGYVTKKYLPALKRLWEQMPAPKWV 120
Mm FpoB         CCGVEMIATGCAHYDTDRFGIIPRNSPRHADVMIISGYVTKKYLPALKRLWDQMPAPKWV 120
Mb FpoB         CCGVEMIAMGCAHYDTDRFGIIPRNSPRQADVMLISGYVTKKYLPALKRLWEQMPSPKWV 120

Ma FpoB         IAMGDCSISGGPFYESYSTVQNIDELFPIDVFIPGCPPRPEALIQGFVELQEKIKAKKDR 180
Mm FpoB         IAMGDCAISGGPFYESYSTVQNIDEIFPIDVYIPGCPPRPEALIQGFVELQEKIKARKDR 180
Mb FpoB         IAFGDCSISGGPFYESYSTVQNIDEIFPIDVFVPGCPPRPEAMLQGFVELQEKIKAKKDL 180

Ma FpoB         GTEY 184
Mm FpoB         GTEY 184
Mb FpoB         GSEY 184
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4. FpoC

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Ma FpoC         MDAMTIIESLTGKFPEAISEAEVESPIRIRAYADKEKAKEVCQYLKDSLQFDHLCSVCGV 60
Mm FpoC         MDARTIIESLTGKFPEAISEAGIESPIRIRAYVDKDKAKEVCEYLKGSLQFDHLCSVSGV 60
Mb FpoC         MDVTEILKSLTGAFPEAISETTAESEIRARAYVEKEKTKEVCQYLKDSLQFDHLCSVCGV 60

Ma FpoC         DYPQRNEQEVVYHIASYDHPVVLMLKARLPRDSPEIESIVPVYWNANWYERETYELFGIF 120
Mm FpoC         DYPQRDELEAVYHIASYDHPVVLMLKARLPRDSPEIESVVSVYWNANWYERETYELYGIF 120
Mb FpoC         DYIKRNELEVVYHIASYNHPVVLTLKAKLPRENPEIESIVSVYWNANWYERETYELFGIL 120

Ma FpoC         FKNHPNLKALVLPEDMLGEWPLRKDYEGFPNRTARNLV 158
Mm FpoC         FKNHPELKPLVLPDDMLGEWPLRKDYEGFPNRTARNLV 158
Mb FpoC         FKNHPNLKPLILPEDMLGEWPLRKDYEGFPNKTARNLV 158
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5. FpoD

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Mb FpoD         MEEKLEPNEMIVHLGPQHPMQPGPFRLNLRLKGETVVDADIELGFIHKGIEKILENKTYL 60
Ma FpoD         MEEMLEPNEMIVHLGPQHPMQPGPFRLNLRLKGETVMDAEVELGFIHKGIEKILENKTYL 60
Mm FpoD         MEEMLESNEMIVHLGPQHPMQPGPFRLNLKLKGETIMDAEVEMGYIHKGIEKILENRTYL 60

Mb FpoD         QGITIVDRICYLVALVNEECFVGCTEKLLGIEPPERSQYIRVILDELTRIQSHLLGMGEF 120
Ma FpoD         QGITIVDRICYLVALTNEECFVGCTEKLLGIEPPERAQYIRVILEELSRLQSHLLGMGEF 120
Mm FpoD         QGITIVDRICYLVALTNEECYVGCVEKLLDIEPPERAQYIRVILEELSRLQSHLLGLGEY 120

Mb FpoD         GEFIGFVSMFMYTIREREEVLSLIDMITGARITHSYLKFGGVRDDLPDGFKEKALSVLNN 180
Ma FpoD         GEFIGFVSMFMYTIKEREDILTLIDMVTGARVTHSYLKFGGVRDDLPEGFKEKALPVLNN 180
Mm FpoD         GEFIGFVSMFMYTIKEREDILTLIDMVTGARVTHSYLRFGGVRDDLPEGFKEKTIPVLNK 180

Mb FpoD         LKKSVDDFEEMFHTDRIYRERTVGVGVLTADVAKNLGVSGPPLRATGVPFDIRKNEPYLV 240
Ma FpoD         LKKVISDYEEMFNSDRIYRERTVGVGVLTADVAKNLGVSGPPLRATGVPFDIRKNEPYLV 240
Mm FpoD         LKKVIRDYEEMFYSDTIYRERTIGIGVLTADEAKSLGVSGPVLRATGVPFDIRKNEPYLV 240

Mb FpoD         YKDLDFKVCTETAGDCFARVQVRINEIRESIYILEQCFDQIPSGPLFPEGSLYGRRTPVM 300
Ma FpoD         YKDLDFKVCTETAGDCFARVQVRLNEMRESIYILEQCLDQIPNGPLFPEGTPYGRRTPVM 300
Mm FpoD         YRDLDFKVCTETAGDCFARVQVRLNEMRESIYIIEQCLDMIPNGPIFPEGTPYGKRTPVM 300
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Fig. S3. Continued.

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Mb FpoD         RVPAGEVFYRVEDPRGEMGMYMISDGSDKPYRVKIRGPYYPTLQALPPLIIGTTVADVAA 360
Ma FpoD         RVPAGEVFHRVEDPRGEMGMYMISDGSDKPYRVKVRGPYYPTLQALPPLIKGTTVADVAA 360
Mm FpoD         RVPAGEVFHRVEDPRGEMGMYMVSDGSDRPYRVKVRGPYYPTLQALPPLIIGTTVADMVS 360

Mb FpoD         ISGSMDGCTSEADR 374
Ma FpoD         ISGSMDGCTSEADR 374
Mm FpoD         ISGSMDGCTSEVDR 374
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6. FpoH

```
Ma FpoH         -MNIMIEIPEFIIPLIPWIRGVVGLVLVGAIFLGAMGAVWLERKLSADIQFRYGPSRVGK 59
Mm FpoH         MTFMAIEIPEFIVPFVPWIRGTVGLVLVGAIFLGGMAAVWIERKLSADIQLRYGPSRVGK 60
Mb FpoH         ---MTVVIPEYITPLIPWVRGIVGLVLIGVIFMGAMGAVWLERKLSADIQTRMGPCRVGK 57

Ma FpoH         FGLLQLVADAIKLFTKEDMRPRNADRLLFDNAPIFMMSSVFLMLVAIPVGAVFINGVEYP 119
Mm FpoH         FGLLQLVADAIKLFTKEDVRPGNADRFLYDNAPVFMLTSLFLMLVAIPVGAVFIDGNLYP 120
Mb FpoH         YGLLQLVADAIKLFTKEDLKPLNADSLLFNNANIFMLGSVFLMLVALPVGAVFINGVEYP 117

Ma FpoH         LAVTEMDISVLYIEAMSAITIFGIFMIAYGSNNKYSLLGAFRNFARMVGYEVPLGITVVS 179
Mm FpoH         LAVTEMDISILFIEAVSAINIFGIFMAAYGSNNKYSLLGAFRNFARMIGYEVPLGIAIVS 180
Mb FpoH         LAVTQMDISVLYIEAVSALSIFGIFMVAYGSNNKYSLLGAFRNFARMVGYEVPLGITVIS 177

Ma FpoH         VAIMTGSLNIVEIASAQG-LLWNIFLQPIGFIVFFIALMADMGRLPFDQNESEEELVAGW 238
Mm FpoH         VAVMTGSLNIIDITSAQGSFVWNIFLQPIGFVVFFIALMADLGRLPFDQNESEEELVAGW 240
Mb FpoH         VAAMTGSLNIVDISTAQG-LHWNIFLQPLGCFVFFVSLMADMGRLPFDQNESEEELIAGW 236

Ma FpoH         ITEYTGMRFGLGFFAEYIHMILGSFLVALLFLGGWNVPAFVANNPVLGLIAPTGFFLLKT 298
Mm FpoH         VTEYTGMRFGLVFFAEYMHMILGSFLVALLFLGGWNVPAFVANNAVLGLIAPTGILLLKT 300
Mb FpoH         ITEYCGMRFGLGFFAEYIHMILGSFLVALLFLGGWNVPGFIANNSFFGIIVPTGFLIVKV 296

Ma FpoH         VLVLMTIIGMRWAVPRFRIDQVVDLSWKRLLPLSLLNLVWAVGLGLYLGA 348
Mm FpoH         VLVLMTIIGMRWAVPRFRIDQVVDMSWKKLLPLSLLNLAWAVGLGLYLGA 350
Mb FpoH         VFVLMVIIGLRWAVPRFRIDQVVDLSWKKLLPLALLNLVWAVGLGLYLGA 346
```

7. FpoI

```
Ma FpoI         MVLKNIKYAVKNIPKKRVTRLCPEVESPLSDRFRGLQILDKSKCIGCGICANTCPNNAIK 60
Mm FpoI         MVLKNIKYALKNIPKERVTRLCPEVESPLSERFRGLQTLDKSKCIGCGICANTCPNSAIK 60
Mb FpoI         MVLKNIKYAIRNITRPPVTRMYPEKQSELSDRFRGLQILDKSKCIGCGICANTCPNAAIK 60

Ma FpoI         IVKAPIAPGSSKQRWFPEIDIGHCLFCGLCIDQCPKGALSSGKEYTKGMVKWAHKDLLMT 120
Mm FpoI         IVKAPIAPGSEKKRWFPQIDIGHCLFCGLCIDQCPKGALSSGKEYCKGMVKWAHKDLLMT 120
Mb FpoI         IVKAPIAPGSTKQRWFPQIDIGHCLFCGLCIDQCPKGALSSGKEYAKGLVKWKHKDLLIT 120

Ma FpoI         PEKLAREVDIKEGDEK 136
Mm FpoI         PEKLAREVDIQEGDER 136
Mb FpoI         PEKLAREVDLEEGDEK 136
```

Fig. S3. Continued.

8. FpoJ

```
Ma FpoJ1        MIGLETVGAALEMAVFGLLAFVTVFFAIFVVIAKDVVRAGLALIMCMFGVAALYILLNAQ 60
Mm FpoJ1        MIDPGTVGAALETAVFGLLALVTVFFAIFVVIAKDVVRAGLALIMCMFGVAGLYILLNAQ 60
Mb FpoJ1        MIELETIGEALKMAVFWVLAISTVFFAVFVVTAKDIVRAGLALIMCMFGIAALYILLNAQ 60

Ma FpoJ1        FLGIIQVLVYIGAIGVLILFAVMLTKRHLGGGSRAD 96
Mm FpoJ1        FLGVIQVLVYIGAIGVLILFAVMLTKREIGGGPRAN 96
Mb FpoJ1        FLGIIQVLVYIGAIGVLILFAVMLTKHEIGGEPGED 96
```

9. FpoJ

```
Ma FpoJ2        MGPVRINRPLALLVSLLFVAVIVTGVFGTSWHTVSELPENPADPSNIQGIGMLIFTQYVV 60
Mm FpoJ2        ---MQINRPLAFLVCLLFVAVVVTGAFGTSWNTVSELPENPADPSNIEGIGMLIFTHFVA 57
Mb FpoJ2        ---MRINRPLAFLVVLLFTAIVVIGAFGTSWNTVSELPQSPADQSNIEGIGMLIFTQYVA 57

Ma FpoJ2        PFEVLSIVLLASLIGAIYMAKGEGNR 86
Mm FpoJ2        PFEVLSIVLLASLIGAIYMAKGEGNR 83
Mb FpoJ2        PFEVLSIVLLASLIGAIYLAKGEGNR 83
```

10. FpoK

```
Ma FpoK         MTAIPLTFYLGLAALLFSIGLYGVMTHKSGIRMIMCIELMLNSANLNLVAFSSYTDTLNG 60
Mm FpoK         MTAIPLTFYLGLAALLFSIGLYGVMTHKSGIRLIMCIELMLNSANLNLVAFSSYTDTLHG 60
Mb FpoK         --MIPLIFYLGLAALLFSIGLYGVMTHKNGIRMIMCIELMLNSANLNLVAFSSYTDTLNG 58

Ma FpoK         QVFAIFSIALAAAEAAVGFAIFMAIYRMHDKINLDELNILRW 102
Mm FpoK         QVFAMFSIALAAAEAAVGFAIFMAIYRMHDKINLDELNILRW 102
Mb FpoK         QVFAVFSIALAAAEAAVGFAILMAIYRMHDKINLNELKSLRW 100
```

11. FpoL

```
Ma FpoL         MVKTALEEFAFLIPLLPALAFAITFFFGKKMPSGGAIVPILAIAASFVISFAITLGLLAN 60
Mm FpoL         MVKTALEEFAFLIPLLPALAFAITFFFGRKMPSGGAIVPILAIAASFVISFAITLGLLAN 60
Mb FpoL         -----MEEFAFLIPLLPALAFVITFFFGRKMPSGGAIVPILAIAASFVISLMITLRLLAN 55

Ma FpoL         PGEVVSQSYSWFAVLDIGILIDPLAAVMLSMVSFVSLLIHIYAVSYMSHDAGKARYFAET 120
Mm FpoL         PEEVISQSYSWFAVLNIGILIDPLAAVMLSMVSFVSLLIHIYAVSYMSHDAGKARYFAET 120
Mb FpoL         PDEVISQSYPWFAVLNIGVLIDPLAAVMLSMVSFVSLLIHIYAVSYMSGDPGEARYFAET 115

Ma FpoL         ALFTAAMLSLVLSDNILQLFVSWELVGLCSYLLIGFWFEKPSAAAAAKKAFLTTRVGDVM 180
Mm FpoL         ALFTAAMLSLVLSDNILQLFVSWELVGLCSYLLIGFWFEKPSAAAAAKKAFLTTRIGDVM 180
Mb FpoL         ALFTAAMLSLVLSDNILQLFVSWELVGLCSYLLIGFWFERPSAAAAAKKAFLTTRIGDVM 175
```

Fig. S3. Continued.

```
Ma FpoL         FLTGIIVLTSDLLKLAGGFQEGVYLLRFDEIFSYIPQLSALQANIFGFEVSHLTIITLLF 240
Mm FpoL         FLTGIIVLTSDLLKVSGGFQDGVYLLRFDEIFSYIPELAALQINILGFEISHLTIITLLF 240
Mb FpoL         FLTGIIVLTSDILKLAGGFQDGTYLLRFDEIFSYIPQLSALQTNIFGFEVSHLTIITLLF 235

Ma FpoL         FGGAVGKSGQFPLHVWLPDAMEGPTTVSALIHAATMVTAGVYLVARTFPMFIAAPGTLMV 300
Mm FpoL         FGGAVGKSGQFPLHVWLPDAMEGPTTVSALIHAATMVTAGVYLVARTFPMFIAAPDSLMV 300
Mb FpoL         FGGAVGKSGQFPLHVWLPDAMEGPTTVSALIHAATMVTAGVYLVARTFPMFIAAPDSLMV 295

Ma FpoL         IAYLGGFTALFAGTMGIVMNDLKRVLAYSTISQLGYMMLALGLGATVGLEAVGVSLFHLI 360
Mm FpoL         VAYFGGFTALFAGTMGIVMNDLKRVLAFSTISQLGYMMLGLGLGTAIGLEAVGISLFHLI 360
Mb FpoL         VAYLGGFTALFAGTMGIVMNDLKRVLAYSTISQLGYMMLGLGLGSAIGLEAIGISLFHLI 355

Ma FpoL         NHAFFKALLFLCAGSVIHAVGTQDMRELGGVGKVMPITAGTMAIAALSLAGFGIPGTSIG 420
Mm FpoL         NHAFFKALLFLCAGSVIHAVGTQDMRELGGVGKVMPITAATMTIAALALAGFGIPGTSIG 420
Mb FpoL         NHAFFKALLFLCAGSVIHAVGTQDMRELGGVRKVMPVTAATMAIAALALAGFGIPGTSIG 415

Ma FpoL         TSGFMSKDPIIENAYLFAEHSGNWIPYIFAIAAALLTSIYIFRLIFMTFAGKPRSDYHGH 480
Mm FpoL         TSGFMSKDPIIEAAYLFGEHSSNWIPYVFSILAALLTSIYIFRLIFMTFTGKPRSNYHGH 480
Mb FpoL         TSGFFSKDAIIEAAYLFGENSNNWIPYAFSIAAALLTSIYIFRLIFMTFTGKPRSDYHGH 475

Ma FpoL         ESPSIMTVPLSILALFALVFGSLTRTGFMNFLEETFTNSFVDLNIGNLAGIGGYELVEAA 540
Mm FpoL         ESPAIMTIPLSILAIFALAFGALTRTGFMEFLEETFTNSFVNLDIGALAGIGENELVAAA 540
Mb FpoL         ESPAIMTIPLSILAIFSLVFGGLTKTGFMNFLEETFANGFVNLNIGGLAALGRNELVGTA 535

Ma FpoL         GHEPVLILWLPLIMAVAGLAIAFVIYYLRVFSLGPIASMKNPIYRLLYKRYYQHEIYTEF 600
Mm FpoL         GHEPLAVLWPPVIVALAGFAIAFVIYYLRAFSLGPLASMKNPIYRLLYNRYYQHQIYTEF 600
Mb FpoL         GSESLFVQWLPMIVAVAGLAVAFVIYYLRIIKLGPLASMKNPVYRLLYKRYYQHKIYTEF 595

Ma FpoL         FSIGIVYGVIAFLTQVVDVIVDSIVEGIGILTVGVGEELRKVQTGVVQTYATVIIAGVSL 660
Mm FpoL         FSIGIVYGIIAFLTQVVDVIIDSVVEGIGIVTVFVGEELRKIQTGVVQTYATALIAGVSL 660
Mb FpoL         FSLGIVYGVIALLSQVLDVIIDSIVEGIGILTVGVSEELRRVQTGVVQTYAIVVIAGVSL 655

Ma FpoL         LIILIKLITEVL 672
Mm FpoL         LIILVKLIMEVL 672
Mb FpoL         LIILVKLIMEVL 667
```

12. FpoM

```
Ma FpoM         MLPVASLLILVPLIFAAVTFFTKTKDQAAGLGLIGSLVTLGLTLYAYLNFDSSTAAMQFY 60
Mm FpoM         MLPVASLLILVPLIFAVVTFFTKTKQLAAGFGFLGSLATLGLTLYAYLNFDSSTAAMQFY 60
Mb FpoM         MLPVASLLILVPLIFAAVTFFTKTKEQAAGLAFLGSLATLGLTLYAYLNFDSSTAAMQFF 60

Ma FpoM         ESVPWVPFLGINYSVGIDGISMPLILLNAIVIPLLILFSWKEDREAPNRFYGLILTMQAA 120
Mm FpoM         ESVSWIPFLGVNYSVGIDGVSMPLILLNAIVIPFMILFTWKEEMESPNRFYGLILTMQAA 120
Mb FpoM         ESIDWIPLLGVKYSVGIDGISMPLILLNAIVIPFLILYSWKEEREDSNRFYGLILTMQAA 120

Ma FpoM         VIGVFVALDFVVFYIFWELTLVPLFFIVNIWGGEKRAHASYKFFIYTHVASLVMLLGIFG 180
Mm FpoM         VIGVFVALDFVVFYIFWELTLVPLFFIVNLWGGANRAHASYKFFIYTHVASLVMLLGIFG 180
Mb FpoM         VIGVFVALDFVVFYIFWELTLIPLFFMVNIWGGEKRAHASYKFFIYTHVASLVMLLGIFG 180
```

Fig. S3. Continued.

```
Ma FpoM         LFYTALHQTGIPTFDIRELIAQFQFFESGLMRDAIFLAILFGFLAKLPTFPFHSWLPDAY 240
Mm FpoM         LFYTALNQTGVPTFDIRELIAQFQFFEPGLMKDGIFLAILFGFLAKLPAFPFHSWLPDAY 240
Mb FpoM         LFYASWQQTGVPTFDIRELVGQFQFLGSGLLRNAIFLSIIFGFLAKLPTFPFHSWLPDAY 240

Ma FpoM         TEAPTAGSVLFILLKIGGYGLFRISLPMLPNTGNPNLMIMMLGLLGSFSIVYGALLALRQ 300
Mm FpoM         SEAPTAGSILFILLKIGGYGLFRISLPMLPNTGSPQLMIMILGLLGSVSILYGALLALRQ 300
Mb FpoM         TEAPTAGSVLFILLKIGGYGLFRISLPMLPNTGNPELMITILGLLGAFSILYGALLALRQ 300

Ma FpoM         KDLKRMIAYSSLSHMGFVTLGSAGLVALSVSGAMFQQFSHGLIMSIMFMSAGAIQTTTGT 360
Mm FpoM         KDLKRMIAYSSLSHMGYVILGSAGLVTLSVSGAMFQQFSHGLIMSIMFMSAGAIQTAAGT 360
Mb FpoM         KDLKRMIAYSSLSHMGYVLLGSAGFVALSVSGAMFQQFSHGLIMSIMFMSAGAIQTSTGT 360

Ma FpoM         RIINDLGGLARKMPMLAVLMMVGFMASLGLPGLTGFIAEFLVLTFSFVNLPGFVLLALLA 420
Mm FpoM         RIINELGGLAKKMPMLTVAMMVGFMASLGLPGLTGFIAEFLVLTFTFTNLPVFVVIALLA 420
Mb FpoM         RIINNLGGLAKKMPTLAVLMMLGFMASLGLPGLTGFIAEFLVLAFSYVNLPGFVLLALLA 420

Ma FpoM         IVITAGYHLWAMQRAMFGVYNEKLGSIRDINSMQVFSMGVIALLVLYFGLNPSPVLNMMI 480
Mm FpoM         IVVTAGYHLWAMQRAMFGVYNEKLGDVRDINSIQVFSMAVIALLVLYFGLNPSPVLDMMI 480
Mb FpoM         IVITAGYHLWAMQRAMFGVYNEKLGDVRDINSLQVFSMAVIALLVVYFGWNPNPVLNMMI 480

Ma FpoM         KNSEAIVSLAAVMGV 495
Mm FpoM         NNSEAIVSLAAGMGV 495
Mb FpoM         TNSEAIVSLAAALGV 495
```

13. FpoN

```
Ma FpoN         MDELMYLAPEIVVVATGLVVLLAGVFLSPRAKNILGYLATLGILAAALFLTVKSFGLLTMQ 60
Mm FpoN         MQEIMYLAPELVLVATGLVILLTGVFLSPQSKNILGYLATLGTLAAIFLTVKSFGLLTME 60
Mb FpoN         MENLMFLAPEIAIAATGLIILFIGVFMSSRTKNVLGYLATLGILAALVLTIQSFG----- 55

Ma FpoN         GFQVGYSIFSEALNIDALSQFFKLVFLVVALIVSIAAIKYNENSDHTEEFYTLMLFATFG 120
Mm FpoN         GFSVQYTIFSETLSIDALSQFFKLVFLAVALIVSIASIKYTENSDHTEEFYTLVLFATFG 120
Mb FpoN         ---TEATMFYGTVSIDALSQFFKLVFLVVALIVSIASIKYNENSDHTEEFYSLVLFATLG 112

Ma FpoN         MMIVASANDLVVLFVAFELASLATYALAGYEKQNPRSLEGAMKYFVIGSVSAALMLFGLS 180
Mm FpoN         MMIVASANDLILLFCAFELASLATFALAGFEKQNARSLEGAMKYFVIGSVSAALMLFGLS 180
Mb FpoN         MMVVASSNDFILLFCAFELASFATYALAGFEKQNPRSLEGAMKYFMMGAVSSALMLFGIS 172

Ma FpoN         FVYGATGTTSIPLIAANPGLLIENPIGLVAVVLLIAGFGFKMALVPFHMWAPDTYQGSPS 240
Mm FpoN         FVYGATGTTSIPLIAQNPGLLTGNPIGIVAIVLLTAGFGFKMALVPFHMWAPDTYQGSPS 240
Mb FpoN         FVYGATGTTSIPMIAENVSLLAENPIGLVAVVLLIAGFGFKMALVPFHMWAPDTYQGSPS 232

Ma FpoN         VVSALLAAGSKKMGFVAAFRIFIVALVALQPDWQFIFTILAVATMTFGNIVAVAQTSVKR 300
Mm FpoN         VVSALLAAGSKKMGFVAAFRVFIIALAALQPDWQFMFTLLAVVTMTFGNVVAVAQTSVKR 300
Mb FpoN         VVSSLLAAGSKKMGFVAAFRVFILALAALQPDWQFAFTILAVVTMTFGNVVAVSQTSVKR 292

Ma FpoN         MLAYSSVAQAGYIAMAFAVMTPVALGGGIMYALAHAFMKAGAFIAAGVVVWMVSQEKTGN 360
Mm FpoN         MLAYSSLAQAGYIAMAFAVMTPVALAGGIMYTLAHAFMKAGAFIAAAAVVWMITSEKTGN 360
Mb FpoN         MLAYSSLAQAGYIAMAFVVMTPMALTGGIFYTLAHAFMKGGAFIAAGAVVWMITTQRTGD 352
```

Fig. S3. Continued.

```
Ma FpoN         LDVPDHLDSFKGLGKRMPLAALSMTVFVFALAGIPPTAGFMAKFVLFSSTIQAGMAWLAV 420
Mm FpoN         LDIPDHLDSFRGLGKRMPLAALCMTVFVFALAGIPPTAGFMAKFVLFSSTIQAGMTWLAV 420
Mb FpoN         LQVPDHLDNFRGLGKRMPLVALCMTVFVFALAGIPLTSGFMAKFVLFSSTIQAGMTWLAV 412

Ma FpoN         IAILNSALSLFYYARLVRYMYFLPPEGK--SVSVPFPYAAALLVAVAGVLVMGIWPEPFV 478
Mm FpoN         IAILNSALSLFYYARLVKYMYFMPPEGKTEKVSIPFPYAAALLVAVAGVLVMGLWPEPFV 480
Mb FpoN         IAILNSALSLFYYARLVRYMYFLPPKGK--KIGLPFPYAAALLLATAGVLVMGLWPEPFL 470

Ma FpoN         ELAMKAAMVLV-- 489
Mm FpoN         ELAMKAAMVLVPF 493
Mb FpoN         QWAMEAAKVLI-- 481
```

14. FpoO

```
Ma FpoO1        MTDCDLCGKAIPTVIPVRVIRPLLKFAYPNGVWKGLCETCLDSAQKTYLEVNKNQPSCRK 60
Mm FpoO         MTDCDLCGKGIPTVIPVRTYPPLLRFAYPEGVWKGLCETCLDSAQKTYLEVNRNHTSCRR 60
Mb FpoO         MTDCDLCGRALPSVIPVRVFRSRLKFAYPEGIWKGLCEACLDSAQETYLSINKDEISCRR 60

Ma FpoO1        GKCALCGDKTGVFPVELQVPDFSKGIVKKDVDLCYRCLKGVDEAYIRHKKEQIEMEH--- 117
Mm FpoO         GKCSLCGSKTGVFSVELQIPDFSKGIVRKDVDVCYRCLKLVDEAYIRYKREQIEQDHEQG 120
Mb FpoO         NKCVLCGKKGRVYPVEIQIPDFSKGVVIKKVNVCTKCLDSINETYIRFKREQIEGSVCE- 119

Ma FpoO1        ---GYH----- 120
Mm FpoO         RIHGHEHVHPH 131
Mb FpoO         --HGHGNVPEH 128
```

15. FpoO1/O2

```
Ma FpoO1        MTDCDLCGKAIPTVIPVRVIRPLLKFAYPNGVWKGLCETCLDSAQKTYLEVNKNQPSCRK 60
Ma FpoO2        MSLLNPK----PDCILVRVVRPLLKFAYPNGVWKDLCETCLDSAQKTYLEANKNQPSCRK 56

Ma FpoO1        GKCALCGDKTGVFPVELQVPDFSKGIVKKDVDLCYRCLKGVDEAYIRHKKEQIEMEHGYH 120
Ma FpoO2        GKCALCGDKTGVFSVELQVPDFVLF---------------------------------- 81
```

Fig. S3. Continued.

A. *mer/fpoF* intergenic region

```
MmfpoFup        TAATTTTTATTACATCTTGCATAATCATTTTTTTTTACGTTATATTTTTAAAA-------- 52
MafpoFup        TAATTTTTATTACATCTTGCATAATCATTTTTTTTTACGTTATATTTTTAAAA-------- 52
MbfpoFup        TAAGTTT-ATTACATCCTGCATAAATTTTTTAATTTCATTGATTTTTTAATTTTTTATTT 59

MmfpoFup        -ACTTTTAAAAAAACATCTTGCCGTTAAGGCAAGCTTATCGAGGCATTGGAGGTAACTGAT 118
MafpoFup        -ACTTTTAAAAAAACATCTTGCCGTTAAGGCAAGCTTATCGAGGCATTGGAGGTAACTGAT 118
MbfpoFup        TATTTTTAAAAAAACATCTTGCCGTTAAGGCAAGCTTATCGAGGCATTGGAGGTAACTGAT 119

MmfpoFup        TG 120
MafpoFup        TG 120
MbfpoFup        TG 121
```

B. *fpoF*/downstream orf intergenic region

```
Mmfpofdn        TGAAGTCCGAAACTTAATTTAAATTCAAAACTTAATTTAAATCCGAAACTTAATTCAAAA 60
MafpoFdn        TGAGACCAGAAGATATATCCAGAT--------------ATATCCAAAA-----------A 35
MbfpoFdn        TGAAATCAGAA--TATATCGAGAAT-------------AAAT----AA------------ 29

Mmfpofdn        AAGAAATATGGTACATTCGAGGTTGGA-TAC--GATG 94
MafpoFdn        GATAACCAGTACAAATTCGAGGTGGAA-TCCGGGATG 71
MbfpoFdn        --TAAATGTATT--GTTGGAGGTTGAAATACGGGATG 62
```

Fig. S4. Alignment of regions flanking *fpoF*. ClustalW was used to align the intergenic region between the methylenetetrahydrosarcinapterin reductase gene (*mer*) and *fpoF* (fpoFup) (*A*) and between *fpoF* and putative cytidylyltransferase gene (fpoFdn) (*B*). Green bases denote conserved amino acid residues. The annotated start and stop codons of genes are in red. The putative ribosome binding sites are in blue. Mb, *M. barkeri*; Mm, *M. mazei*; Ma, *M.acetivorans*.

Table S1. Pairwise percent identities* of *M. barkeri* (Mb) Fpo, *M. acetivorans* (Ma) Fpo, *M. mazei* (Mm) Fpo†, and *E. coli* (Ec) Nuo predicted protein sequences

	Mb FpoF	Ma FpoF	Mm FpoF	Ec NuoF
Mb FpoF	—	86	88	Absent
Ma FpoF		—	89	
Mm FpoF			—	
	Mb FpoA	Ma FpoA	Mm FpoA	Ec NuoA
Mb FpoA	—	91	92	36
Ma FpoA		—	99	35
Mm FpoA			—	34
	Mb FpoB	Ma FpoB	Mm FpoB	Ec NuoB
Mb FpoB	—	88	85	37
Ma FpoB		—	92	38
Mm FpoB			—	40
	Mb FpoC	Ma FpoC	Mm FpoC	Ec NuoC
Mb FpoC	—	82	79	24
Ma FpoC		—	89	30
Mm FpoC			—	20
	Mb FpoD	Ma FpoD	Mm FpoD	Ec NuoD
Mb FpoD	—	91	83	35
Ma FpoD		—	90	36
Mm FpoD			—	37
	Mb FpoH	Ma FpoH	Mm FpoH	Ec NuoH
Mb FpoH	—	83	77	38
Ma FpoH		—	86	39
Mm FpoH			—	39
	Mb FpoI	Ma FpoI	Mm FpoI	Ec NuoI
Mb FpoI	—	85	83	29
Ma FpoI		—	91	29
Mm FpoI			—	20
	Mb FpoJ	Ma FpoJ	Mm FpoJ	EcNuoJ
Mb FpoJ	—	81	81	21
Ma FpoJ		—	88	23
Mm FpoJ			—	21
	Mb FpoK	Ma FpoK	Mm FpoK	Ec NuoK
Mb FpoK	—	92	90	38
Ma FpoK		—	97	37
Mm FpoK			—	35
	Mb FpoL	Ma FpoL	Mm FpoL	Ec NuoL
Mb FpoL	—	87	88	34
Ma FpoL		—	89	33
Mm FpoL			—	34
	Mb FpoM	Ma FpoM	Mm FpoM	Ec NuoM
Mb FpoM	—	88	85	30
Ma FpoM		—	88	31
Mm FpoM			—	30
	Mb FpoN	Ma FpoN	Mm FpoN	Ec NuoN
Mb FpoN	—	80	81	31
Ma FpoN		—	88	31
Mm FpoN			—	32
	Mb FpoO	Ma FpoO1	Ma FpoO2	Mm FpoO
Mb FpoO	—	63	55	58
Ma FpoO1		—	79	80
Ma FpoO2			—	66
Mm FpoO				—

*ClustalW was used for determination of percent identity.
†Locus tags for genes encoding Fpo subunits are given in Fig. S1.

Table S2. *M. barkeri* Fusaro strains used in this study

Strain	Genotype	Source or Construction
WWM85	Δ*hpt*::*PmcrB*-ϕC31*int*-attP	(8)
WWM86	Δ*hpt*::*PmcrB*-ϕC31*int*-attB	(8)
WWM71	Δ*hpt*::*PmcrB*-ϕC31*int*-attB, Δ*fpoA-O*	Deletion of *fpo* by markerless exchange with pDK4 in WWM86
WWM123	Δ*hpt*::*PmcrB*-ϕC31*int*-attP, Δ*fpoF*	Deletion of *fpoF* by markerless exchange with pDK13 in WWM85
WWM116	Δ*hpt*::*PmcrB*-ϕC31*int*-attP, Δ*freAEGB*	Deletion of *fre* by markerless exchange with pGK6 in WWM85
WWM122	Δ*hpt*::*PmcrB*-ϕC31*int*-attB, Δ*frhADGB*::*pac-hpt*	Deletion of *frh* with ApaI/NotI-digested pAMG81 in WWM86
WWM108	Δ*hpt*::*PmcrB*-ϕC31*int*-attB, Δ*fpoA-O*, Δ*frhADGB*::*pac-hpt*	Deletion of *frh* with ApaI/NotI-digested pAMG81 in WWM71
WWM145	Δ*hpt*::*PmcrB*-ϕC31*int*-attP, Δ*fpoF*, Δ*frhADGB*::*pac-hpt*	Deletion of *frh* with ApaI/NotI-digested pAMG81 in WWM123

Table S3. Plasmids used in this study

Plasmid	Description and/or Construction	Reference
pMP44	Vector containing a *pac-hpt* cassette used to delete genes from *M. barkeri Fusaro* chromosome using the markerless exchange method	(7)
pDK4	SpeI/XmaI-digested *fpo* upstream PCR product amplified using primers LfpoA and RfpoA1 and NotI/XmaI-digested *fpo* downstream PCR product amplified using primers LfpoO and RfpoO were ligated to SpeI/NotI-digested pMP44	This study
pDK13	SpeI/XmaI-digested *fpoF* upstream PCR product amplified using primers FusfpoF(us)for and FusfpoF(us)rev and NotI/XmaI-digested *fpoF* downstream PCR product amplified using primers FusfpoF(ds)for and FusfpoF(ds)rev were ligated to SpeI/NotI-digested pMP44	This study
pGK6	AscI/PstI-digested *fre* upstream and downstream fusion PCR product amplified using primers freupfor, freuprev, frednfor, and frednrev and ligated to MluI/NsiI-digested pMP44	This study
pJK301	Vector containing a *pac-hpt* cassette used to delete genes from *M. barkeri Fusaro* chromosome using double-homologous recombination-mediated gene replacement method	(14)
pAMG78	ApaI/XhoI-digested *frh* upstream PCR product amplified using primers Fusfrhupfor and Fusfrhuprev and ligated to ApaI/XhoI-digested pJK301	This study
pAMG81	SpeI/NotI-digested *frh* downstream PCR product amplified using primers Fusfrhdnfor and Fusfrhdnrev and ligated to Spe/NotI-digested pAMG78	This study

Table S4. Primers used in this study

Primer	Sequence*	Added Sites
LfpoA	ACTAGTGAAGTGAACCCTCGCCTCTT	SpeI
RfpoA1	GGATCCCCCGGGCATATGTATCACCTATTAAAGTGCAGC	BamHI/XmaI/NdeI
LfpoO	AAGCTTCCCGGGTGAATTTGAGTAAAGCTGCATTTTG	HindIII/XmaI
RfpoO	CTCGAGGCGGCCGCCCTACTAATGTTGGCATTGACG	XhoI/NotI
FusfpoF(us)for	GGCGCGCCACTAGTGAATCGAATTTGTGCCGAGCGA	AscI/SpeI
FusfpoF(us)rev	GGCGCGCCCCCGGGTTAGTTACCTCCAACACCTT	AscI/XmaI
FusfpoF(ds)for	GGCGCGCCCCCGGGAATCAGAATATATCGAGAATAA	AscI/XmaI
FusfpoF(ds)rev	GGCGCGCCGCGGCCGCTTTTTAAATCCGATTTTCAC	AscI/NotI
freupfor	GGCGCGCCAAATCCGATGCATTCTCTGC	AscI
freuprev	CAGTGTAAATAACAAAATAGTTTTTCGCTGCCTCGTTTTCTATTTGGTG	None
frednfor	CACCAAATAGAAAACGAGGCAGCGAAAAACTATTTTGTTATTTACACTG	None
frednrev	GGCGCGCCCTGCAGCCCGTAAACCATCCAACATC	AscI/PstI
Fusfrhupfor	GGCGCGCCCTTAAGGGGCCCTCCGTTGTCCTTCTTTCCAC	AscI/AflII/ApaI
Fusfrhuprev	GGCGCGCCACTAGTCTCGAGCAATTGTATGCCTCGTTTTCGATTT	AscI/SpeI/XhoI/MfeI
Fusfrhdnfor	GGCGCGCCACTAGTTAAAACTCCTCTTTTTTTGTCAGCG	AscI/SpeI
Fusfrhdnfor	GGCGCGCCGCGGCCGCTGTTGCGAGTTGTTCAATCC	AscI/NotI
QPCRrpoA1for	GGCTTCGCTGCAAGACATG	None
QPCRrpoA1rev	CCCGAAGTGTCCAGGACATT	None
QPCRfpofor	CCTTCTCCGAAATGGGTCATC	None
QPCRfporev	AAACGGGCCGCCACTAA	None

*The added restriction sites are underlined.