An XoxF-type Methanol Dehydrogenase from the anaerobic Methanotroph '*Candidatus* Methylomirabilis oxyfera'

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Running Head: XoxF methanol dehydrogenase from *Methylomirabilis oxyfera*

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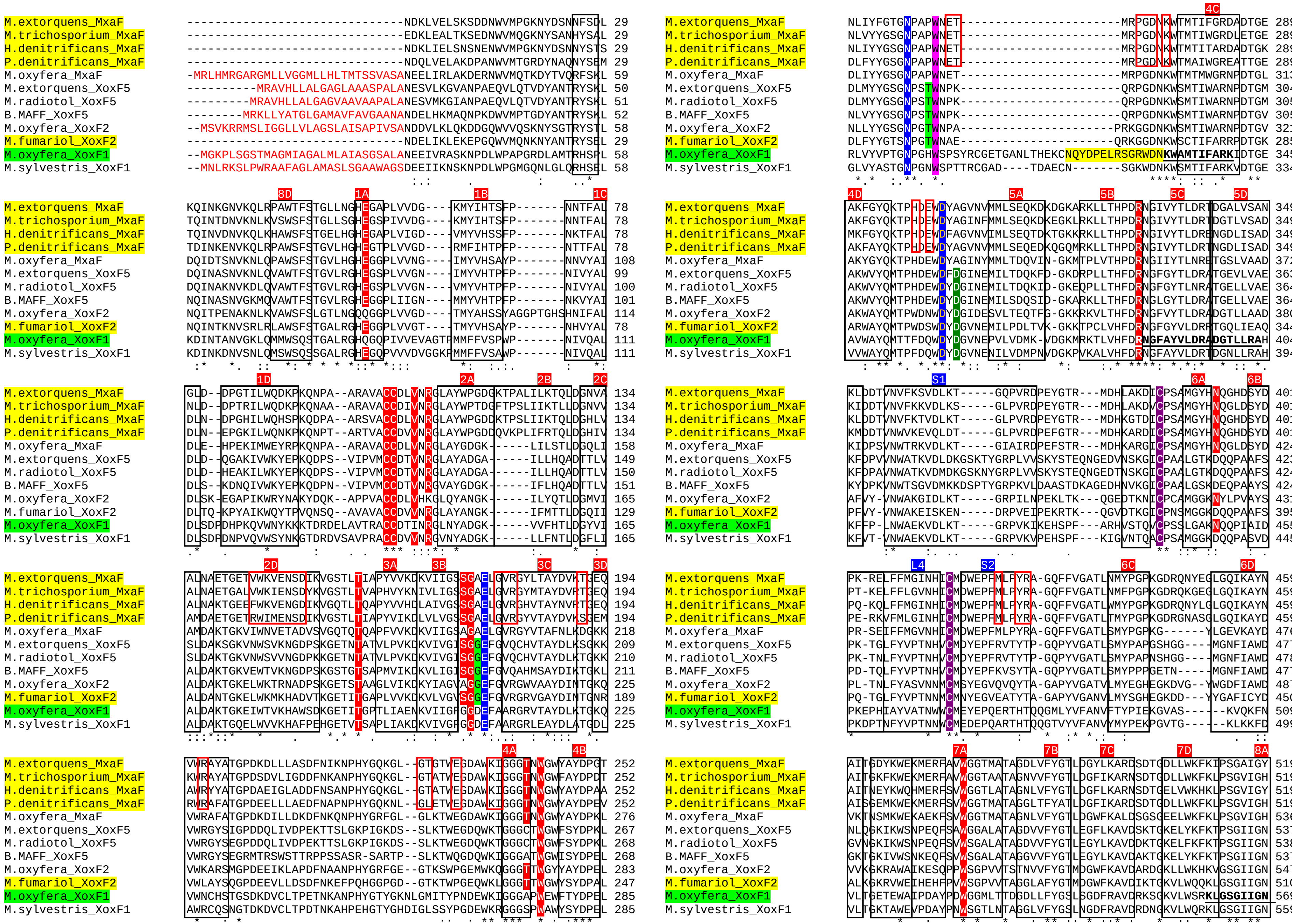
A



M.extorquens_MxaF
M.trichosporium_MxaF
H.denitrificans_MxaF
P.denitrificans_MxaF
M.oxyfera_MxaF
M.extorquens_XoxF5
M.radiotol_XoxF5
B.MAFF_XoxF5
M.oxyfera_XoxF2
M.fumariol_XoxF2
M.oxyfera_XoxF1
M.sylvestris_XoxF1

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                                      8B               S3                             8C
M.extorquens_MxaF         PMTYT-HKGTQYVAIYYGVGGWPGVGLVFDLA--DPTAGLGAVG-AFKK-LANYTQMGGG 574
M.trichosporium_MxaF      PIVYQ-HKGVEYVAILYGVGGWPGVGLVFDLQ--DPTAGLGAVG-AFKQ-LANYTQMGGG 574
H.denitrificans_MxaF      PMTYE-HKGVQYIAVMSGVGGWPGVGLVFDLQ--DPTAGLGAVG-AFKN-LQNYTQMGGS 574
P.denitrificans_MxaF      PMTYK-HDGRQYVAIMYGVGGWPGVGLVFDLA--DPTAGLGSVG-AFKR-LQEFTQMGGG 574
M.oxyfera_MxaF            PITYE-HNGKQYVAILYGVGGWPGVGLVFDLN--DPTAGLGSVG-AFKE-LAKYTKMGGG 591
M.extorquens_XoxF5        VMTYE-HKGKQHVAVLSGVGGWAGIGLAAGLT--DPNAGLGAVG-GYAA-LSSYTNLGGQ 592
M.radiotol_XoxF5          VMTYQ-HKGKQYVGVLSGVGGWAGIGLAAGLT--DPNAGLGAVG-GYAA-LSQYTNLGGQ 593
B.MAFF_XoxF5              VTTYE-NNGKQYVAVLSGVGGWAGIGLAAGLT--DPTAGLGAVG-GYAA-LSNYTALGGT 592
M.oxyfera_XoxF2           PITYKGPDGKQYVAVYSGVGGWFGATVSLDLPPDDPTAALGGVNAAYKSGLPMATTKGGT 607
M.fumariol_XoxF2          PITFLGPDKKQYVAVYSGVGGWFGIAVAQNLPPDDPYAGLGAVGVAYQAGLPKATTVGGE 570
M.oxyfera_XoxF1           PITYK-IKGKQYVSVLAGIGGWIGLPVTAELDFDDKYGAIGATAMAKLTGLDKIPQ-GGV 627
M.sylvestris_XoxF1        PIAYK-IKGHEYISVFAGIGGWIGLPAVAGLDLEDKFGAIGSTALTKVIGLNKIPQ-GGA 617
                            .:   .  :::.:  *:*** *      *   *  ..:*..       *   .  **

M.extorquens_MxaF         VVVFSLDGKGPYDDPNVGEWKSAAK- 599
M.trichosporium_MxaF      VMVFSLNGNGPYDDVNLGEYKAN--- 597
H.denitrificans_MxaF      LEVFSLDGKNPYDDVNVGEYEKG--- 597
P.denitrificans_MxaF      VMVFSLDGESPYSDPNVGEYAPGEPT 600
M.oxyfera_MxaF            LMVFALPEGR---------------- 601
M.extorquens_XoxF5        LTVFSLPNN----------------- 601
M.radiotol_XoxF5          LTVFALPN------------------ 601
B.MAFF_XoxF5              LTVFSLPQQ----------------- 601
M.oxyfera_XoxF2           LYVFGL-------------------- 613
M.fumariol_XoxF2          LYVFALE------------------- 577
M.oxyfera_XoxF1           LYTFRID------------------- 634
M.sylvestris_XoxF1        LYTFRVPDKEATAH------------ 631
                          : .* :
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B

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M.extorquens_MxaI         YDGTKCKAAGNCWEPKPGFPEKIAGSKYDPKHDPKELNKQADSIKQMEERNKKRVENFKK 60
H.denitrificans_MxaI      YDGTHCKAPGNCWEPKPGFPEKIAGSKYDPKHDPKELNKQVESRKGEEERNANRAEHFKK 60
Methylophilus_MxaI        YDGQNCKEPGNCWENKPGYPEKIAGSKYDPKHDPVELNKQEESIKAMDARNAKRIANAKS 60
P.denitrificans_MxaI      YDGTNCKAPGNCWEPKPDYPAKVEGSKYDPQHDPAELSKQGESLAVMDARNEWRVWNMKK 60
M.oxyfera_MxaI            YDGTKCKEPGACWEPKPGYPARVAGSKYDPKHDPKEVAKQELAHKAMDERNEKRWKHFQE 60
                          *** :** .* *** **.:* :: ******:*** *: **  :     : **  *  : :.

M.extorquens_MxaI         TGKFEYDVAKISAN--------- 74
H.denitrificans_MxaI      TGKWVYDVKKIQ----------- 72
Methylophilus_MxaI        SGNFVFDVK-------------- 69
P.denitrificans_MxaI      TGKFEYDVKKIDGYDETKAPPAE 83
M.oxyfera_MxaI            TGVWKYDVK-------------- 69
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Fig S1 Structure-based multiple amino acid sequence alignment of selected methanol dehydrogenase (**A**) large and (**B**) small subunits. (**A**) Beta sheets found in the structures of the large subunits are placed in black boxes. The eight W-shaped β propellers (1-8) composed of four β strands each (A-D) that structure the proteins (1-4) are marked red with white lettering on top of the boxes. Conserved amino acids involved in the binding of PQQ and of Ca^{2+} are highlighted red and blue with white lettering, respectively. Asp301 found in the crystal structure of the XoxF-type MDH from *Methylacidiphilum fumariolicum* SolV (M.fumariol_XoxF2) (5) to additionally coordinate the catalytic lanthanide ($La3^{+}$, Ce^{3+}) is marked dark green (white lettering); other amino acid substitutions (Thr259 and Gly171) related with the accommodation of the larger lanthanide ion are marked light green (black lettering). Asp299 (M.fumariol_XoxF2 numbering), which is a catalytic key enzyme is marked blue (orange lettering). Other structural and catalytic key amino acids (1-4) are highlighted in various colors. S1-S3 and L4 represent variable sequences that confer enzyme specificity to PQQ-containing enzymes (4, 6). Amino acids that bind the small subunit (MxaI) in MxaFI-MDHs are boxed red. Predicted N-terminal leader sequences are printed in red; leader sequences have been omitted in the MxaF- and XoxF-type methanol dehydrogenases with known crystal structures. Proteins with known crystal structures are marked yellow in their coding names. M.oxyfera_XoxF1 (DAMO_0124) identified in this paper to constitute the large MDH subunit is marked light green. The peptide sequence used for the generation of polyclonal antibodies is marked yellow (black lettering). Protein names, bacterial species and protein accession numbers are the following (PDB codes in parentheses): M.extorquens_MxaF, *Methylobacterium extorquens* AM1, YP_001641590 (1H4I); M.trichosporium_MxaF, *Methylosinus trichosporium* OB3b, EFH02986 (1G72); H.denitrificans_MxaF, *Hyphomicobium denitrificans* ATCC 51888, ADJ23135 (2D0V); P.denitrificans_MxaF, *Paracoccus denitrificans* PD1222, ABL71076 (1LRW); M.oxyfera_MxaF, DAMO_0112; M.extorquens_XoxF5, *Methylobacterium extorquens* PA1, ACS39584; M.radiotol_XoxF5, *Methylobacterium radiotolerans* JCM 2831,ACB22519; B.MAFF_XoxF5, Bradyrhizobium diazoefficiens USDA 110, NP_772853; M.oxyfera_XoxF2, DAMO_0134; M.fumariol_XoxF2, *M fumariolicum* SolV, CCG91656 (4MAE); M.sylvestris_XoxF1, *Methylocella silvestris* BL2, ACK52058. The XoxF5 proteins included in the sequence alignment have an established function as methanol dehydrogenases and contain a REE (40-42). (**B**) Amino acid sequences of small subunits. N-terminal leader sequences (MAAQRIGLRLSIGLSLLAAASSAVA) of M.oxyfera_MxaI (DAMO_0115) and of small units with known crystal structures have been omitted for clarity. Amino acids implicated in the binding of cyt c_L (7) are highlighted by red boxes. Other protein names and accession numbers of small subunits are as follows (PDB codes in parentheses): M.extorquens_MxaF, *Methylobacterium extorquens* AM1 (1H4I); *H. denitrificans* ATCC 51888, ADJ23132 (2D0V); Methylophilus_MxaI, Methylophilus methylotrophus W3A1/ *Methylosinus trichosporium* OB3b (1G72); *P. denitrificans* PD1222, ABL71079 (1LRW). Alignments were made by the ClustalW program. In (A) and (B), peptides identified in MDH from *M. oxyfera* by MALDI-TOF MS are underlined and printed in bold.

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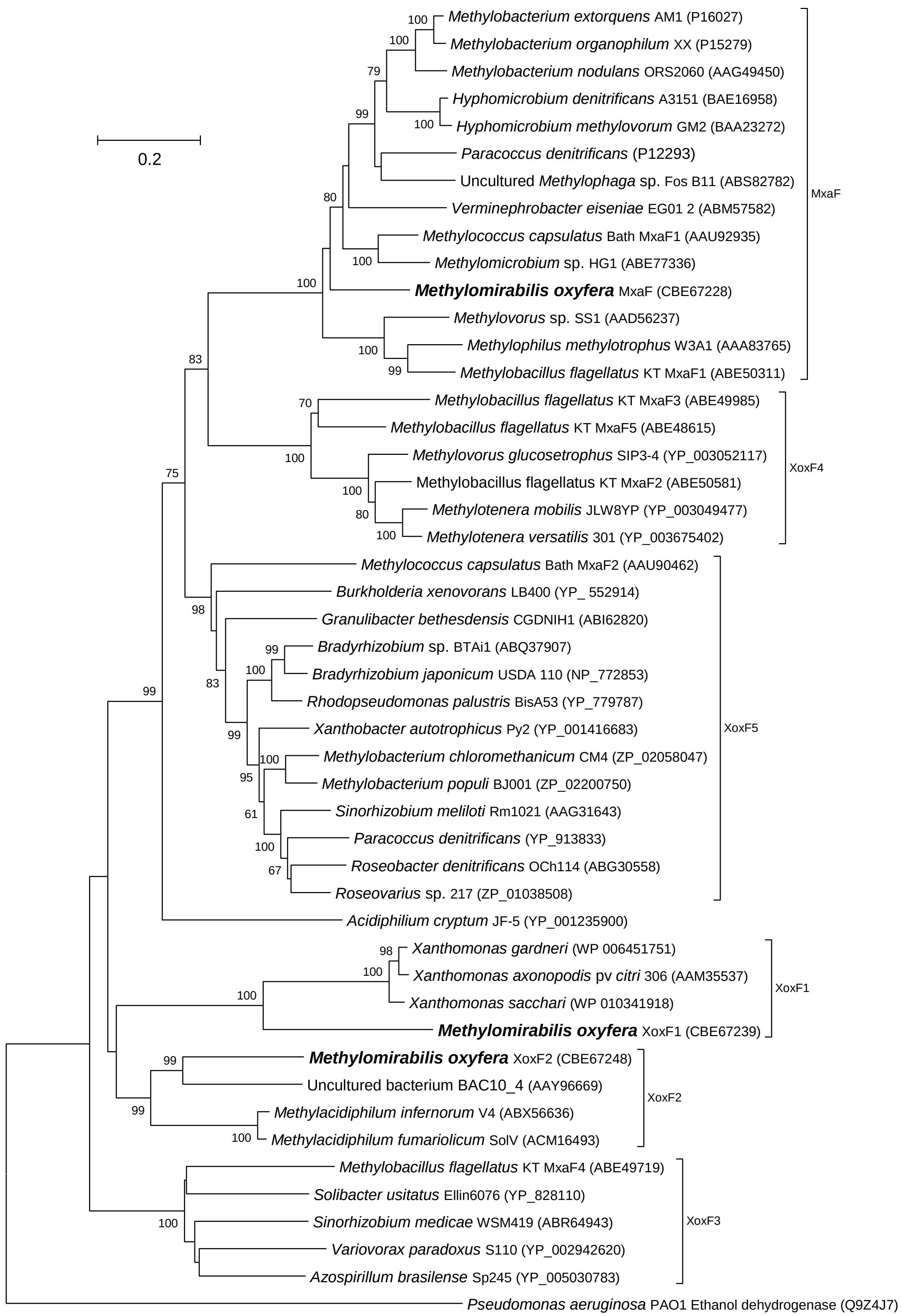

0.2
Methylobacterium extorquens AM1 (P16027)
Methylobacterium organophilum XX (P15279)
Methylobacterium nodulans ORS2060 (AAG49450)
Hyphomicrobium denitrificans A3151 (BAE16958)
Hyphomicrobium methylovorum GM2 (BAA23272)
Paracoccus denitrificans (P12293)
Uncultured Methylophaga sp. Fos B11 (ABS82782)
Verminephrobacter eiseniae EG01 2 (ABM57582)
Methylococcus capsulatus Bath MxaF1 (AAU92935)
Methylomicrobium sp. HG1 (ABE77336)
Methylomirabilis oxyfera MxaF (CBE67228)
Methylovorus sp. SS1 (AAD56237)
Methylophilus methylotrophus W3A1 (AAA83765)
Methylobacillus flagellatus KT MxaF1 (ABE50311)
MxaF
Methylobacillus flagellatus KT MxaF3 (ABE49985)
Methylobacillus flagellatus KT MxaF5 (ABE48615)
Methylovorus glucosetrophus SIP3-4 (YP_003052117)
Methylobacillus flagellatus KT MxaF2 (ABE50581)
Methylotenera mobilis JLW8YP (YP_003049477)
Methylotenera versatilis 301 (YP_003675402)
XoxF4
Methylococcus capsulatus Bath MxaF2 (AAU90462)
Burkholderia xenovorans LB400 (YP_ 552914)
Granulibacter bethesdensis CGDNIH1 (ABI62820)
Bradyrhizobium sp. BTAi1 (ABQ37907)
Bradyrhizobium japonicum USDA 110 (NP_772853)
Rhodopseudomonas palustris BisA53 (YP_779787)
Xanthobacter autotrophicus Py2 (YP_001416683)
Methylobacterium chloromethanicum CM4 (ZP_02058047)
Methylobacterium populi BJ001 (ZP_02200750)
Sinorhizobium meliloti Rm1021 (AAG31643)
Paracoccus denitrificans (YP_913833)
Roseobacter denitrificans OCh114 (ABG30558)
Roseovarius sp. 217 (ZP_01038508)
XoxF5
Acidiphilium cryptum JF-5 (YP_001235900)
Xanthomonas gardneri (WP 006451751)
Xanthomonas axonopodis pv citri 306 (AAM35537)
Xanthomonas sacchari (WP 010341918)
Methylomirabilis oxyfera XoxF1 (CBE67239)
XoxF1
Methylomirabilis oxyfera XoxF2 (CBE67248)
Uncultured bacterium BAC10_4 (AAY96669)
Methylacidiphilum infernorum V4 (ABX56636)
Methylacidiphilum fumariolicum SolV (ACM16493)
XoxF2
Methylobacillus flagellatus KT MxaF4 (ABE49719)
Solibacter usitatus Ellin6076 (YP_828110)
Sinorhizobium medicae WSM419 (ABR64943)
Variovorax paradoxus S110 (YP_002942620)
Azospirillum brasilense Sp245 (YP_005030783)
XoxF3
Pseudomonas aeruginosa PAO1 Ethanol dehydrogenase (Q9Z4J7)

Fig S2 Evolutionary relationships of MxaF and XoxF type methanol dehydrogenases. The evolutionary history was inferred using the Neighbor-Joining method (8). The optimal tree with the sum of branch length = 9.69298341 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (9). Values above 60 are shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Dayhoff matrix based method (10) and are in the units of the number of amino acid substitutions per site (scale bar). The analysis involved 48 amino acid sequences; protein accession numbers are mentioned in parentheses. All positions containing gaps and missing data were eliminated. There were a total of 526 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (11). The five clades of XoxF proteins along with MxaF proteins proposed by Chistoserdova (12) are bracketed at the right side of the figure. Note that our analysis suggests one more clade represented by an XoxF-type of MDH from *Acidiphilium cryptum* JF-5 (YP_001235900).

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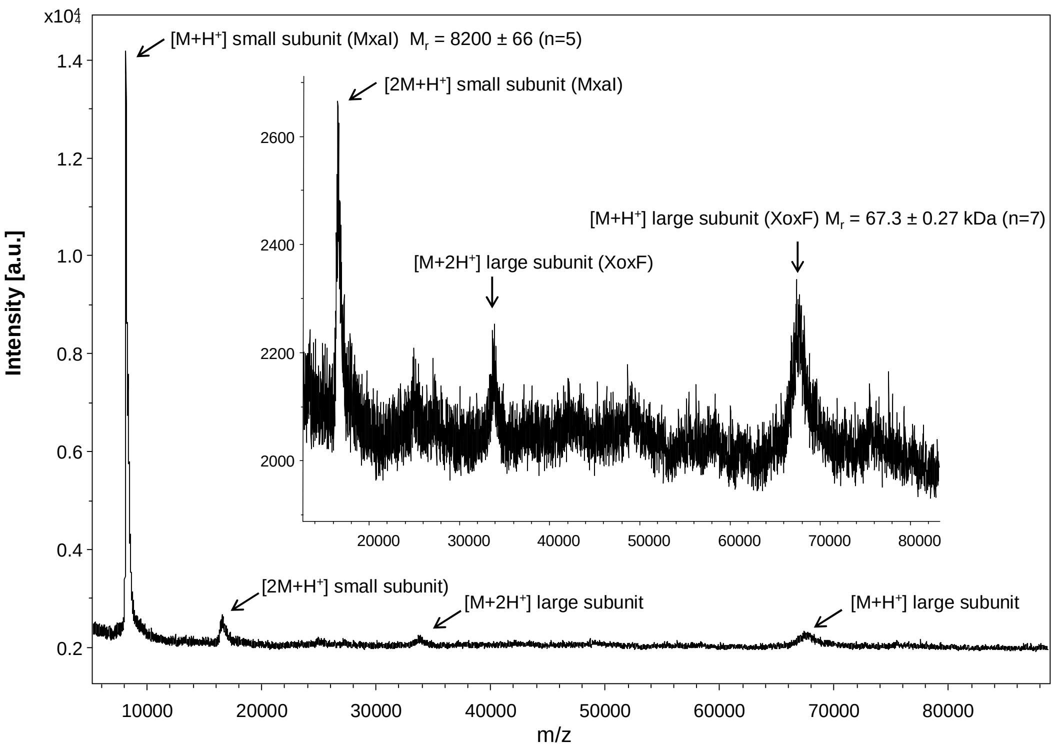

FIG S3 Linear MALDI-TOF MS of methanol dehydrogenase from *M. oxyfera*. The analysis was performed on the as-isolated protein as described in Materials and Methods. Annotated peaks are indicated by arrows. The inset shows the 13000-8300 *m/z* region in more detail.

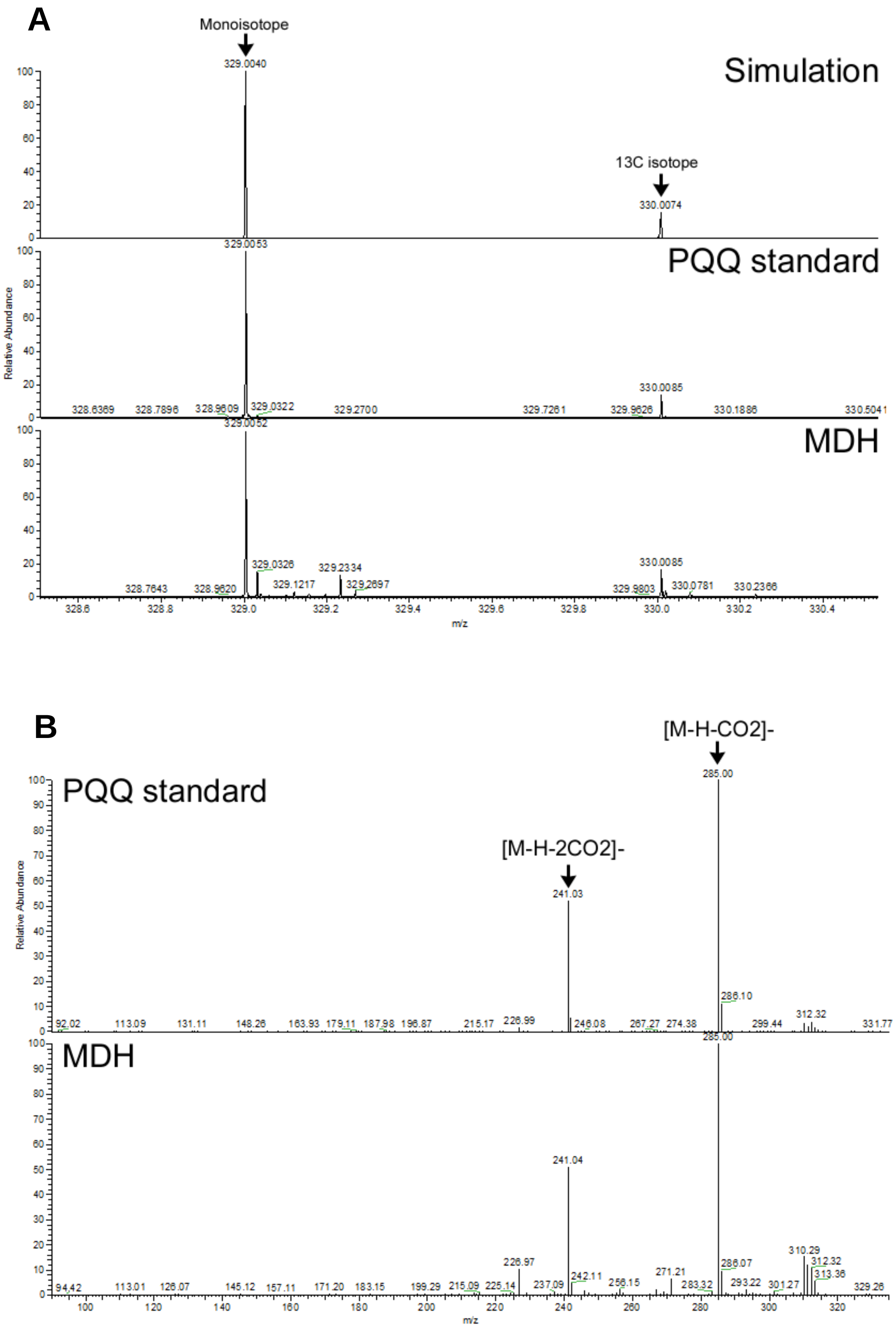

FIG S4 Mass spectroscopic identification of PQQ in the purified methanol dehydrogenase. A, Simulated and acquired FT-ICR MS spectra of PQQ *m/z* 329 [M-H]- precursor ion. B, linear ion trap CID fragmentation spectra of *m/z* 329 [M-H]- for the PQQ standard and the enzyme preparation. Diagnostic fragmentation peaks are annotated in the Figure.