

**An XoxF-type Methanol Dehydrogenase from the anaerobic Methanotroph ‘*Candidatus*
Methyloirabilis oxyfera’**

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Running Head: XoxF methanol dehydrogenase from *Methyloirabilis oxyfera*

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The Netherlands

A

M.extorquens_MxaF -----NDKLVELSKSDNNVMPGKNYDSNFSIDL 29
M.trichosporium_MxaF -----EDKLEALTKSEDNVMWQKKNYSANHYSL 29
H.denitrificans_MxaF -----NDKLIELSNENMVMPPGKNYDSNHYSTS 29
P.denitrificans_MxaF -----NDQLVELAKDPANVWMTGRDYNAQVNSFM 29
M.oxyfera_MxaF -MRLHMRGARGMLLVGGMLLHMTSSVASANEELIRLAKDERNVMWQKTYDVTQRFPSKL 59
M.extorquens_XoxF5 -----MRAVHLHLGAGLAASAPALANEVSLKGVANPAEQVLQTVDYANTRYSL 50
M.radiotol_XoxF5 -----MRAVHLHLGAGVAAPVAPALANEVSMVKGIANPAEQVLQTVDYANTRYSL 51
B.MAFF_XoxF5 -----MRKLLYATGLGMAVFAVGAANENDELHKMAQNPMDVMWPTGQDYANTRYSL 52
M.oxyfera_XoxF2 -----MSVKKRMSLIGLLVLGASLAISAPIVSAANDVLLKQKDDGQVWVQSKNYSGTRYSL 58
M.fumariol_XoxF2 -----NDEILKLEKEPQGWQMNKNYANTRYSL 29
M.oxyfera_XoxF1 --MGKPLSGSTMAGMIAGALMLAASGSALANEELIVRASKNPDLWPAQGRDLAMTRHSL 58
M.sylvestris_XoxF1 --MNLRLKSLPWRAPAGLAMASLSGAAWAGSDEEIIKNSKNPDLWPGMQNLGLQRHSL 58

M.extorquens_MxaF KQINKGNVQLRPAWTFSTGLLNGHGGAPLVVDG---KMYIHTSFP-----NNTFAL 78
M.trichosporium_MxaF TQINTDNVKNLWVWSFSSTGLLSGHEGSPIVVDG---KMYIHTSFP-----NNTFAL 78
H.denitrificans_MxaF TQINVDNVKQLKHAWFSSTGLLHGHGGAPLVVDG---VMYVHSSFP-----NKTFFAL 78
P.denitrificans_MxaF TDINKENNVKQLRPAWFSSTGLVLRHGGTPLVVDG---RMFIHTFP-----NNTFAL 78
M.oxyfera_MxaF DQIDTSNVKNLQPAWFSSTGLVLRHGGTPLVVDG---IMYVHSAFP-----NNTVYAI 108
M.extorquens_XoxF5 DQINASNVLNQLVAVTFSTGLVLRHGGSPPLVGN---IMYVHTFP-----NIVYAL 99
M.radiotol_XoxF5 DQINAKNVKDLQVAVTFSTGLVLRHGGSPPLVGN---VMYVHTFP-----NIVYAL 100
B.MAFF_XoxF5 NQINASNVMKQVAVTFSTGLVLRHGGPLIIGN---MMYVHTFP-----NKVYAI 101
M.oxyfera_XoxF2 NQITPENAKNLKVAWFSSTGLTLNGHGGPLVVDG---TMYAHSSVAGGPTGHSNIFAL 114
M.fumariol_XoxF2 NQINTKNVSRLLVAWFSSTGLALRGGGGPLVVDG---TMYVHSAFP-----NHVYAL 78
M.oxyfera_XoxF1 KDINTANVKLLMMSWSSTGLALRGGGGPVIIVVAVGTFMMFFVSPWP-----NIVQAL 111
M.sylvestris_XoxF1 KDINKDNVSNLQMSWSSTGLALRGGGGPVIIVVAVGTFMMFFVSAWP-----NIVQAL 111

M.extorquens_MxaF GLD--DPGTLILWQDKPKQNPAA--ARAVACCDIVNRCCLAYWPDGGKTPALILKTLQDGNVA 134
M.trichosporium_MxaF NLD--DPTRIWLQDKPKQNA--ARAVACCDIVNRCCLAYWPTDGTTPSLIKTLQDGNVA 134
H.denitrificans_MxaF LND--DPGHLLWQHSKQDPA--ARAVACCDIVNRCCLAYWPGDGTTPSLIKTLQDGHVLA 134
P.denitrificans_MxaF DLN--EPGKILWQNKPKQNP--ARTVACCDIVNRCCLAYWPGDGVKPLIFRTQDGHVLA 134
M.oxyfera_MxaF DLE--HPEKIMWYRFPKQNPAA--ARAVACCDIVNRCCLAYLADGK-----LILSLOTQLL 158
M.extorquens_XoxF5 DLD--GQAKILWYKYPKQDPS--VIVPVMCCDIVNRCCLAYADGA-----ILLHQADITL 149
M.radiotol_XoxF5 DLD--HEAKILWYKYPKQDPS--VIVPVMCCDIVNRCCLAYADGA-----ILLHQADITL 150
B.MAFF_XoxF5 DLS--KNDQILWYKYPKQDPN--VIVPVMCCDIVNRCCLAYADGK-----ILFHQADITL 151
M.oxyfera_XoxF2 DLSK--EGAPIKWRYNAKYDQK--APPVACCDIVNRCCLAYANGK-----ILYQTLDSMVI 165
M.fumariol_XoxF2 DLSDQ--KPYAIKWQYTPVQNSQ--AVAVACCDIVNRCCLAYANGK-----IPMTLTDQIIL 129
M.oxyfera_XoxF1 DLSDEHPKQVWNYKKTDRDELAVTRAACCDIVNRCCLAYADGK-----VVFHTLTDGYVI 165
M.sylvestris_XoxF1 DLSDEDPNPQVMSYKKTDRDVSAPVRAACCDIVNRCCLAYADGK-----LLENTLDBFLI 165

M.extorquens_MxaF ALNAEETGETVWVKVENSIDKVGSTLTIAPVVKIKVVIIGSGAGLGVRYGYLTAYDVRTCEQ 194
M.trichosporium_MxaF ALNAEETGAIWVKIENSIDYKVGSTLTIAPVHYKNIIVLIGSGAGLGVRYGYMTAYDVRTCEQ 194
H.denitrificans_MxaF ALNAEETGEEFWKVENGDIKVGSTLTIAPVYVHILAVIGSGAGLGVRYGYVTAAYDVRTCEQ 194
P.denitrificans_MxaF AMDAETGETRWIENSIDKVGSTLTIAPVVIKILVIGSGAGLGVRYGYVTAAYDVRTSEM 194
M.oxyfera_MxaF AMDAKTGVVWVETADVVSQGTQIQAPFVVKIKVVIIGSAGAGLGVRYGYVTAAYDVRTDGGK 218
M.extorquens_XoxF5 SLDAKSGKVNWSVNGDPSKGETNTATLPLVKIKVVIIGSGGDFGQVCHVTAYDLRSGSK 210
M.radiotol_XoxF5 SLDAKTGVNWSVNGDPSKGETNTATLPLVKIKVVIIGSGGDFGQVCHVTAYDLRSGTK 209
B.MAFF_XoxF5 ALDAKTGVKVTNKGNDPSKGSTGTAAMVVIKIKVVIIGSGGDFGQVCHVTAYDLRSGTK 211
M.oxyfera_XoxF2 ALDAKTGKELWKTNRADPSKGETSTAAGLVIKIKVVIIGSAGDFGVRGVAAYDINTCKQ 225
M.fumariol_XoxF2 ALDANTGKELWKMHADVTKGETTIGAPLVIKIKVVIIGSGGDFGVRGVAAYDINTGNR 189
M.oxyfera_XoxF1 ALDAKTGKEIWTVKHAWSDKGETTIGHTLIIANVKVVIIGSGDFGVAARGRTVAYDLRSGTK 225
M.sylvestris_XoxF1 ALDAKTGQELWVVKHAFPEHGETVTSAPLIAKIKVVIIGSGDFGVAARGRLAYDLRSGTDL 225

M.extorquens_MxaF VWRAYATGPDKDLLASDFNINKNPHYQKGL--GTGTWEGDAWKIIGGNWGWYAYDPDT 252
M.trichosporium_MxaF KWRAYATGPDSDVLIGDDFNANKNPHYQKGL--GTATWEGDAWKIIGGNWGWYAYDPDT 252
H.denitrificans_MxaF AVRKYATGPDABEGLADDNSANPHYQKGL--GTATWEGDAWKIIGGNWGWYAYDPA 252
P.denitrificans_MxaF RWRAYATGPDDELLAEDFNANPHYQKGL--GLTWTWEGDAWKIIGGNWGWYAYDPDV 252
M.oxyfera_MxaF VWRAYATGPDKDLLDKDFNKQNPYGRFGL--GLKTWEGDAWKIIGGNWGWYAYDPA 276
M.extorquens_XoxF5 VWRGYSIGPDDQLIVDPEKTTSLGKPIIGKDS--SLKTWEGDQWKTGGGCTWGWYAYDPKL 267
M.radiotol_XoxF5 VWRGYSIGPDDQLIVDPEKTTSLGKPIIGKDS--SLKTWEGDQWKTGGGCTWGWYAYDPKL 268
B.MAFF_XoxF5 VWRGYSIGRMTRSWSTTRPSSASR-SARTP--SLKTWEGDQWKTGGGATWGWYAYDPKL 268
M.oxyfera_XoxF2 VVKARS-MGPDDEIKLAPDFNANPHYGRFGE--GTKSWPGEWKKGGCTWGWYAYDPKL 283
M.fumariol_XoxF2 VWLAYSQGPDEEVLDSDFNKEFPYHGGYGD--GKTWPGEQWKLGGCTWGWYAYDPAL 247
M.oxyfera_XoxF1 VVNCHTSIPGDDVCLTPETNKANPHYQKGLMGIYSPNDEWKIIGGAPDEWSTYDPDL 285
M.sylvestris_XoxF1 AWRQCSNGTDKDVCLTPTDINKAHPHGTGYGHDIGLSSYPGDEWKRGGGSPWAWYAYDPKL 285

M.extorquens_MxaF NLIYFGTGNPAPWNET-----MRPGDKNMTITIFGRDADTGE 289
M.trichosporium_MxaF NLIYVYGGSNPAPWNET-----MRPGDKNMTITIWGRDLETGE 289
H.denitrificans_MxaF NLIYVYGGSNPAPWNET-----MRPGDKNMTITITARDADDTGK 289
P.denitrificans_MxaF DLFYVYGGSNPAPWNET-----MRPGDKNMTIAMIWGREATTGE 289
M.oxyfera_MxaF DLIYVYGGSNPAPWNET-----MRPGDKNMTITMWRGNADDTGL 313
M.extorquens_XoxF5 DLMYVYGGSNPSTWPK-----QRPGDNKWSMTIWARNEADDTG 304
M.radiotol_XoxF5 DLMYVYGGSNPSTWPK-----QRPGDNKWSMTIWARNEADDTG 305
B.MAFF_XoxF5 NLIYVYGGSNPSTWPK-----QRPGDNKWSMTIWARNEADDTG 305
M.oxyfera_XoxF2 NLIYVYGGSNPSTWPK-----PRKGGDNKWSMTIWARNEADDTG 321
M.fumariol_XoxF2 DLFYVYGGSNPSTWPK-----QRKGGDNKWSMTIWARNEADDTG 385
M.oxyfera_XoxF1 RLIVYVYGGSNPSTWPK-----NOYDEPLRSGRWNNKMTIFARKADDTGE 245
M.sylvestris_XoxF1 GLVYASTGNPSTWPK-----TDAECN-----SGKWDNWSMTIWARNEADDTGE 334

M.extorquens_MxaF AKFGYQKTPHEDNRYAGVNVMLSEQDKKDGKARKLLTHPDRNGIIVTLDRDAGALVSAN 349
M.trichosporium_MxaF AKFGYQKTPHEDNRYAGVNVMLSEQDKKDGKARKLLTHPDRNGIIVTLDRDAGTGLVSAD 349
H.denitrificans_MxaF MKFGYQKTPHEDNRYAGVNVMLSEQDKKDGKARKLLTHPDRNGIIVTLDRDAGGLISAD 349
P.denitrificans_MxaF AKFGYQKTPHEDNRYAGVNVMLSEQDKKDGKARKLLTHPDRNGIIVTLDRDAGGLISAD 349
M.oxyfera_MxaF AKYGYQKTPHEDNRYAGVNVMLTDQVFN-GKMTPLVTHPDRNGIIVTLDRDAGLSAAD 372
M.extorquens_XoxF5 AKWVYQKTPHEDNRYAGVNVMLTDQVFN-GKDRKLLTHPDRNGIIVTLDRDAGLVVAE 363
M.radiotol_XoxF5 AKWVYQKTPHEDNRYAGVNVMLTDQVFN-GKDRKLLTHPDRNGIIVTLDRDAGLVVAE 364
B.MAFF_XoxF5 AKWVYQKTPHEDNRYAGVNVMLTDQVFN-GKDRKLLTHPDRNGIIVTLDRDAGLVVAE 364
M.oxyfera_XoxF2 AKWAYQKTPHEDNRYAGVNVMLTDQVFN-GKDRKLLTHPDRNGIIVTLDRDAGLVVAE 380
M.fumariol_XoxF2 ARWAYQKTPHEDNRYAGVNVMLTDQVFN-GKDRKLLTHPDRNGIIVTLDRDAGLVVAE 344
M.oxyfera_XoxF1 AVWAYQKTPHEDNRYAGVNVMLTDQVFN-GKDRKLLTHPDRNGIIVTLDRDAGLVVAE 404
M.sylvestris_XoxF1 VVWAYQKTPHEDNRYAGVNVMLTDQVFN-GKDRKLLTHPDRNGIIVTLDRDAGLVVAE 404

M.extorquens_MxaF KLDLDTNVFVKSVDLKT---GQPVREPEYGT---MDHLAKDIPSAMGYHNOGHDSYD 401
M.trichosporium_MxaF KLDLDTNVFVKSVDLKS---GLPVRPEYGT---MDHLAKDIPSAMGYHNOGHDSYD 401
H.denitrificans_MxaF KLDLDTNVFVKSVDLKT---GLPVRPEYGT---MDHLAKDIPSAMGYHNOGHDSYD 401
P.denitrificans_MxaF KMDDTNVWKEVQLDT---GLPVRPEYGT---MDHLAKDIPSAMGYHNOGHDSYD 401
M.oxyfera_MxaF KLDPSNVWTRKVDLKT---GATRDPEFSTR---MDHLAKDIPSAMGYHNOGHDSYD 424
M.extorquens_XoxF5 KFDPPVWATKVLDDKGSKYGRPLVSKYSTQNGEDNKSGLKPAALGTQDQPAFAS 423
M.radiotol_XoxF5 KFDPPVWATKVLDDKGSKYGRPLVSKYSTQNGEDNKSGLKPAALGTQDQPAFAS 424
B.MAFF_XoxF5 KYDPKPNWATKVLDDKGSKYGRPLVSKYSTQNGEDNKSGLKPAALGTQDQPAFAS 424
M.oxyfera_XoxF2 KYDPKPNWATKVLDDKGSKYGRPLVSKYSTQNGEDNKSGLKPAALGTQDQPAFAS 424
M.oxyfera_XoxF2 AFVY--VWAKGIDLKT---GRPILNPEKLT---QGEDTKNIPCSAMGGKQYLPVAYS 431
M.fumariol_XoxF2 PFVY--VWAKELSKEN---DRPVEPEKRT---QGVDTKKNIPCSAMGGKQYLPVAYS 395
M.oxyfera_XoxF1 KFFP--VWAKVVDLKT---GRPVKIKESHPF---ARHVTQVPSLGAKNQQLIAD 455
M.sylvestris_XoxF1 KFTV--VWAKVVDLKT---GRPVKIKESHPF---KIGVNTQVPSAMGGKQYLPVAYS 445

M.extorquens_MxaF PK-RELFPMGINNHCMWDEPFFMLFYRA-GQFFVYGATLNMYPGKGRDQNYEGLGQIKAYN 459
M.trichosporium_MxaF PT-KEFLFGLVNHCMWDEPFFMLFYRA-GQFFVYGATLNMYPGKGRDQNYEGLGQIKAYN 459
H.denitrificans_MxaF PE-KQFLFGLVNHCMWDEPFFMLFYRA-GQFFVYGATLNMYPGKGRDQNYEGLGQIKAYN 459
P.denitrificans_MxaF PQ-RKFLFGLVNHCMWDEPFFMLFYRA-GQFFVYGATLNMYPGKGRDQNYEGLGQIKAYN 459
M.oxyfera_MxaF PR-SEFLFPMGINNHCMWDEPFFMLFYRA-GQFFVYGATLNMYPGKGRDQNYEGLGQIKAYN 476
M.extorquens_XoxF5 PK-TGLFVYVPTNHCMWDEPFFMLFYRA-GQFFVYGATLNMYPGKGRDQNYEGLGQIKAYN 477
M.radiotol_XoxF5 PK-TGLFVYVPTNHCMWDEPFFMLFYRA-GQFFVYGATLNMYPGKGRDQNYEGLGQIKAYN 478
B.MAFF_XoxF5 PL-TGLFVYVPTNHCMWDEPFFMLFYRA-GQFFVYGATLNMYPGKGRDQNYEGLGQIKAYN 477
M.oxyfera_XoxF2 PD-TNLFYASVNNHCMWDEPFFMLFYRA-GAPYVYGATLNMYPGKGRDQNYEGLGQIKAYN 487
M.fumariol_XoxF2 PQ-TGLFVYVPTNHCMWDEPFFMLFYRA-GAPYVYGATLNMYPGKGRDQNYEGLGQIKAYN 450
M.oxyfera_XoxF1 PKPEPHLAVATNWHCMWDEPFFMLFYRA-GQFFVYGATLNMYPGKGRDQNYEGLGQIKAYN 509
M.sylvestris_XoxF1 PKDPTNFYVPTNHHCMWDEPFFMLFYRA-GQFFVYGATLNMYPGKGRDQNYEGLGQIKAYN 459

M.extorquens_MxaF AITGDKYKWEKMERFAVGGTMAATAGDLVYFGTLDGVLKARDSDTQDLLWFKPLPSGAI 519
M.trichosporium_MxaF AITGDKYKWEKMERFAVGGTMAATAGDLVYFGTLDGVLKARNSDTQDLLWFKPLPSGAI 519
H.denitrificans_MxaF AITGDKYKWEKMERFAVGGTMAATAGDLVYFGTLDGVLKARNSDTQDLLWFKPLPSGAI 519
P.denitrificans_MxaF AITGDKYKWEKMERFAVGGTMAATAGDLVYFGTLDGVLKARNSDTQDLLWFKPLPSGAI 519
M.oxyfera_MxaF AITGDKYKWEKMERFAVGGTMAATAGDLVYFGTLDGVLKARNSDTQDLLWFKPLPSGAI 519
M.extorquens_XoxF5 NLTGKIKWNSPEQFASVGGALATAGDVFYFGTLDGVLKARNSDTQDLLWFKPLPSGAI 537
M.radiotol_XoxF5 GVNKIKWNSPEQFASVGGALATAGDVFYFGTLDGVLKARNSDTQDLLWFKPLPSGAI 538
B.MAFF_XoxF5 NLTGKIKWNSPEQFASVGGALATAGDVFYFGTLDGVLKARNSDTQDLLWFKPLPSGAI 537
M.oxyfera_XoxF2 VVKGKRAWLIKESQFASVGGPVVTSINVVYFGTLDGVLKARNSDTQDLLWFKPLPSGAI 547
M.fumariol_XoxF2 ALKGRKRWLIEHPFASVGGPVVTSINVVYFGTLDGVLKARNSDTQDLLWFKPLPSGAI 510
M.oxyfera_XoxF1 VLTGKIKWNSPEQFASVGGALATAGDVFYFGTLDGVLKARNSDTQDLLWFKPLPSGAI 569
M.sylvestris_XoxF1 VLTGKIKWNSPEQFASVGGALATAGDVFYFGTLDGVLKARNSDTQDLLWFKPLPSGAI 559

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|------------------------|--------------------------|--------------|---|-------------|------------|
| | | 8B | | 8C | |
| M.extorquens_MxaF | EMTYT-HKGTQYVAIYY | EVGGW | EVGLVPDLA--DPTAGLGAVG-AFKK-LANYTQ | MGGG | 574 |
| M.trichosporium_MxaF | EVVYQ-HKGVQYVAILY | EVGGW | EVGLVPDLQ--DPTAGLGAVG-AFKK-LANYTQ | MGGG | 574 |
| H.denitrificans_MxaF | EMTYE-HKGVQYIAVMS | EVGGW | EVGLVPDLQ--DPTAGLGAVG-AFKK-LQNYTQ | MGGS | 574 |
| P.denitrificans_MxaF | EMTYK-HDGRQYVAIMY | EVGGW | EVGLVPDLA--DPTAGLGSVG-AFKK-LQEFTQ | MGGG | 574 |
| M.oxyfera_MxaF | HITYE-HNGKQYVAILY | EVGGW | EVGLVPDLN--DPTAGLGSVG-AFKE-LAKYTH | MGGG | 591 |
| M.extorquens_XoxF5 | VMTYE-HKGGQHVAVLS | EVGGW | AGIGLAAGLT--DPNAGLGAVG-GYAA-LSSYTH | LGGG | 592 |
| M.radiotol_XoxF5 | VMTYQ-HKGGQYVAVLS | EVGGW | AGIGLAAGLT--DPNAGLGAVG-GYAA-LSQYTH | LGGG | 593 |
| B.MAFF_XoxF5 | VTTYE-MNGKQYVAVLS | EVGGW | AGIGLAAGLT--DPTAGLGAVG-GYAA-LSNYTH | LGGT | 592 |
| M.oxyfera_XoxF2 | HITKGPDKQYVAVYS | EVGGW | GATVSLDLPPDDPTAALGGVNAAYKSGLPMAH | TKGGT | 607 |
| M.fumariol_XoxF2 | HITFLGPDKKQYVAVYS | EVGGW | GIAVAQNLPPDDPYAGLGAVGVAYQAGLPKHT | TVGGE | 570 |
| H.oxyfera_XoxF1 | HITYK-IKGGQYVSVLA | EVGGW | GLPVTAEALDFDDKYGAIGATAMAKLTGLDKIPQ | -GGV | 627 |
| M.sylvestris_XoxF1 | HITAYK-IKGEHYISVFA | EVGGW | GLPAVAGLLEDEKFGAIGSTALTKVIGLNKIPQ | -GGA | 617 |
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| M.extorquens_MxaF | VVVFSLD | SKGPYDDPNVGEWKSAAK-- | 599 |
| M.trichosporium_MxaF | VMVFLN | GNNGPYDDVNLGEYKAN--- | 597 |
| H.denitrificans_MxaF | LEVFSLD | GKNPYDDVNVGEYKKG--- | 597 |
| P.denitrificans_MxaF | VMVFSLD | GESPYSDPNVGEYAPGEPT | 600 |
| M.oxyfera_MxaF | LMVFALP | EGR----- | 601 |
| M.extorquens_XoxF5 | LTVFSLP | NN----- | 601 |
| M.radiotol_XoxF5 | LTVFALP | N----- | 601 |
| B.MAFF_XoxF5 | LTVFSLP | QQ----- | 601 |
| M.oxyfera_XoxF2 | LYVFG | L----- | 613 |
| M.fumariol_XoxF2 | LYVFALE | ----- | 577 |
| H.oxyfera_XoxF1 | LYTFRID | ----- | 634 |
| M.sylvestris_XoxF1 | LYTFRVPE | KEATAH----- | 631 |
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|----------------------|--|------------|-----------|----------------------|-----------|---------------|-----|
| M.extorquens_MxaI | YDGTKCKAAGNCWEPKPGFPEKIAGSKYDP | RHD | PK | ELNKQADS | IKQMEERNK | KRVENFKK | 60 |
| H.denitrificans_MxaI | YDGTHTCKAPGNCWEPKPGFPEKIAGSKYDP | RHD | PK | ELNKQVESR | KGEEERNAN | RAEHFKK | 60 |
| Methylophilus_MxaI | YDGNCKEFGNCWENKPGYPEKIAGSKYDP | RHD | PK | VELNKQ | EESIKAM | DARNAKRIANAKS | 60 |
| P.denitrificans_MxaI | YDGTNCKAPGNCWEPKPDYPKAVGSKYDP | PHD | PK | PAELSKQGESL | AVMDARNE | WRVWNMKK | 60 |
| M.oxyfera_MxaI | YDGTKCKE FGACWEPKPGYPARVAGSKYDP | RHD | PK | EVAKQ ELAHKAM | DERNEKR | WKHPQE | 60 |
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|----------------------|-------------------------|----|
| M.extorquens_MxaI | TGKFEYDVAKISAN----- | 74 |
| H.denitrificans_MxaI | TGKWYDVKKIQ----- | 72 |
| Methylophilus_MxaI | SGNFVFDVK----- | 69 |
| P.denitrificans_MxaI | TGKFEYDVKKIDGYDETKAPPAE | 83 |
| M.oxyfera_MxaI | TGVVKY DVK----- | 69 |

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 (La^{3+} , 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, *Hyphomicrobium 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 sylvestris* 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 (MAAQRIGLRISIGLSLLAAASSAVA) 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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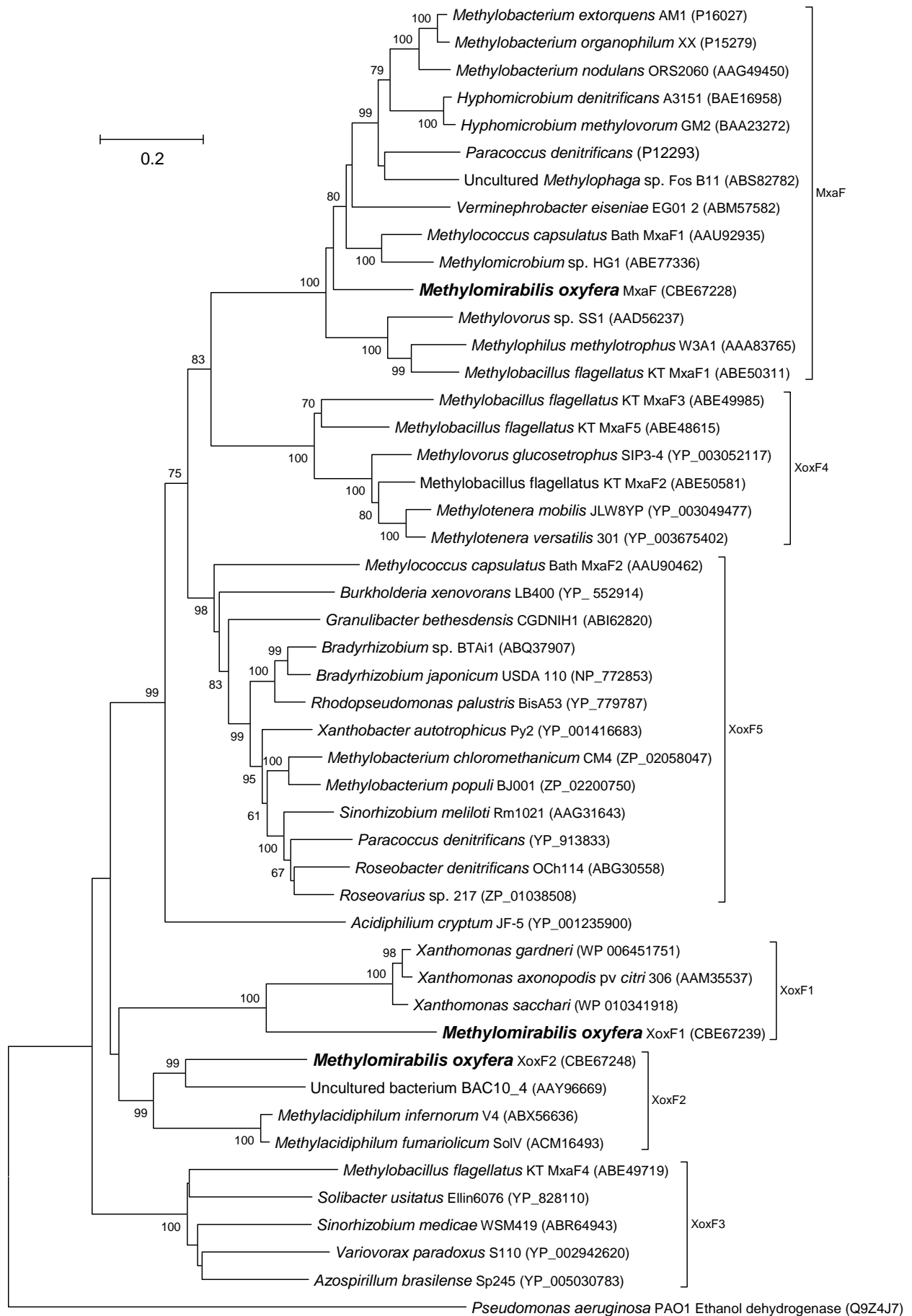


Fig S2 Evolutionary relationships of MxaF and XoxF type methanol dehydrogenases. The evolutionary history was inferred using the Neighbor-Joining method (8). The optimal tree with the sum of branch length = 9.69298341 is shown. The percentage of replicate trees in which the associated taxa clustered 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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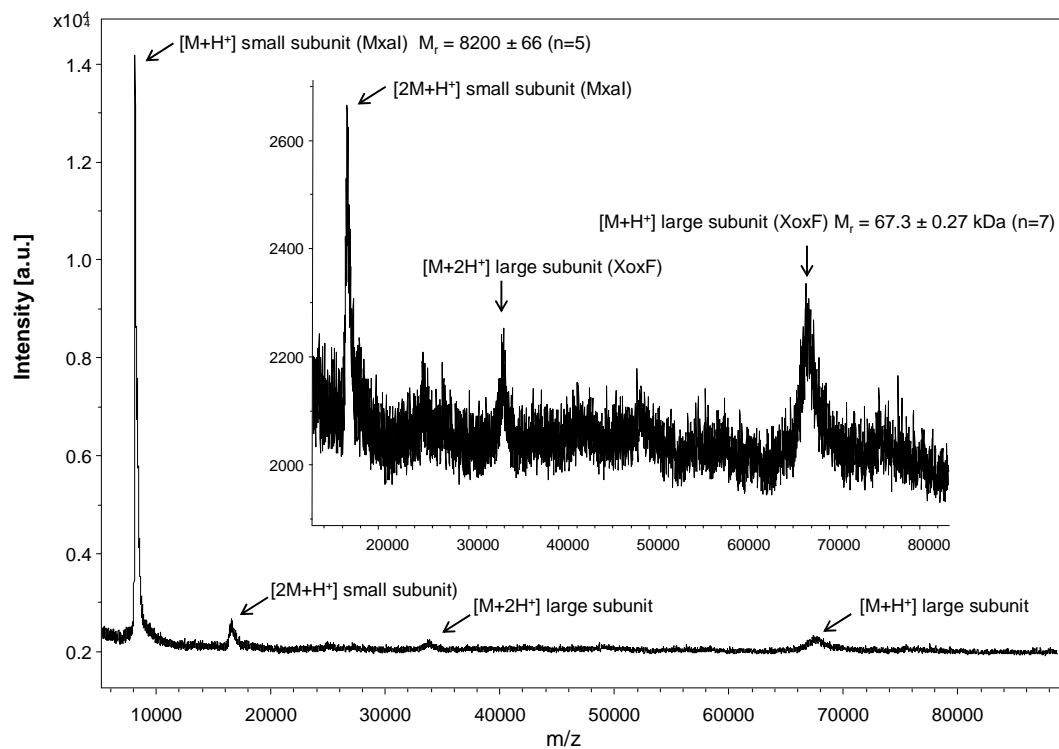


FIG S3 Linear MALDI-TOF MS of methanol dehydrogenase from *M. oxyfera*. The analysis was performed on the as-isolated protein as described in Materials and Methods. Annotated peaks are indicated by arrows. The inset shows the 13000-8300 m/z region in more detail.

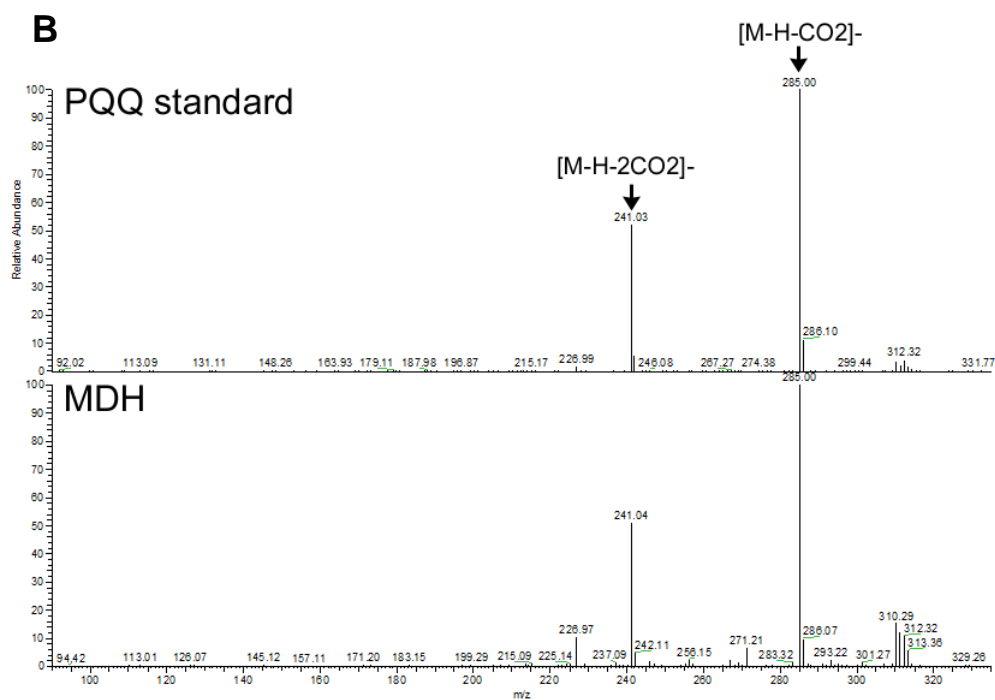
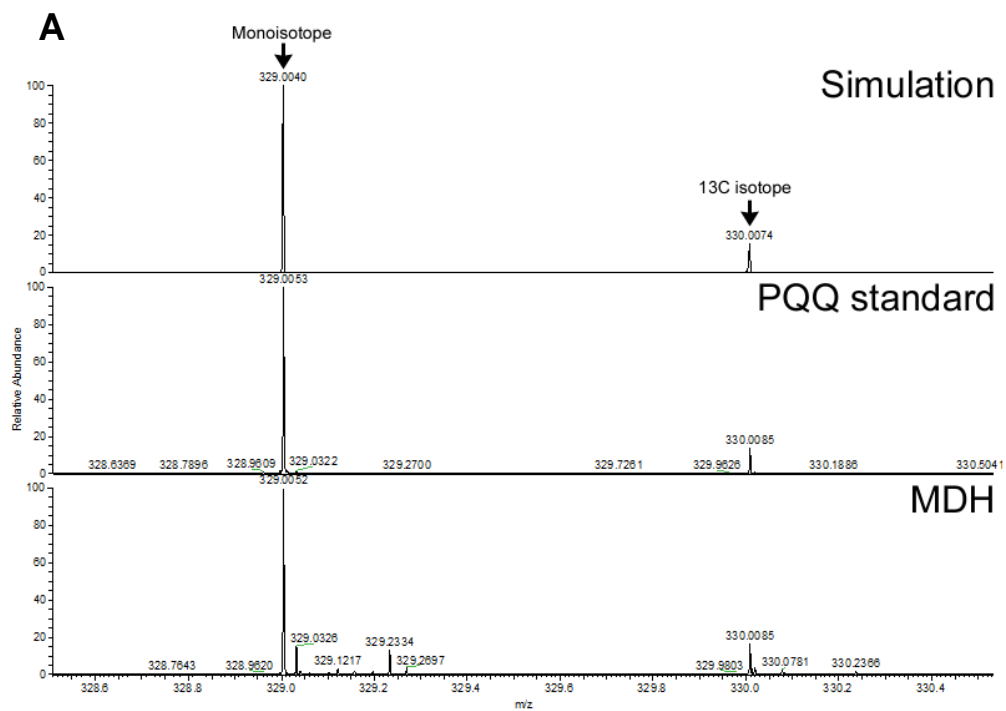


FIG S4 Mass spectroscopic identification of PQQ in the purified methanol dehydrogenase. A, Simulated and acquired FT-ICR MS spectra of PQQ m/z 329 $[\text{M}-\text{H}]^-$ precursor ion. B, linear ion trap CID fragmentation spectra of m/z 329 $[\text{M}-\text{H}]^-$ for the PQQ standard and the enzyme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