SUPPLEMENTARY MATERIAL

Biocatalytic Oxidations of Substrates through Soluble Methane Monooxygenase from *Methylosinus sporium* 5

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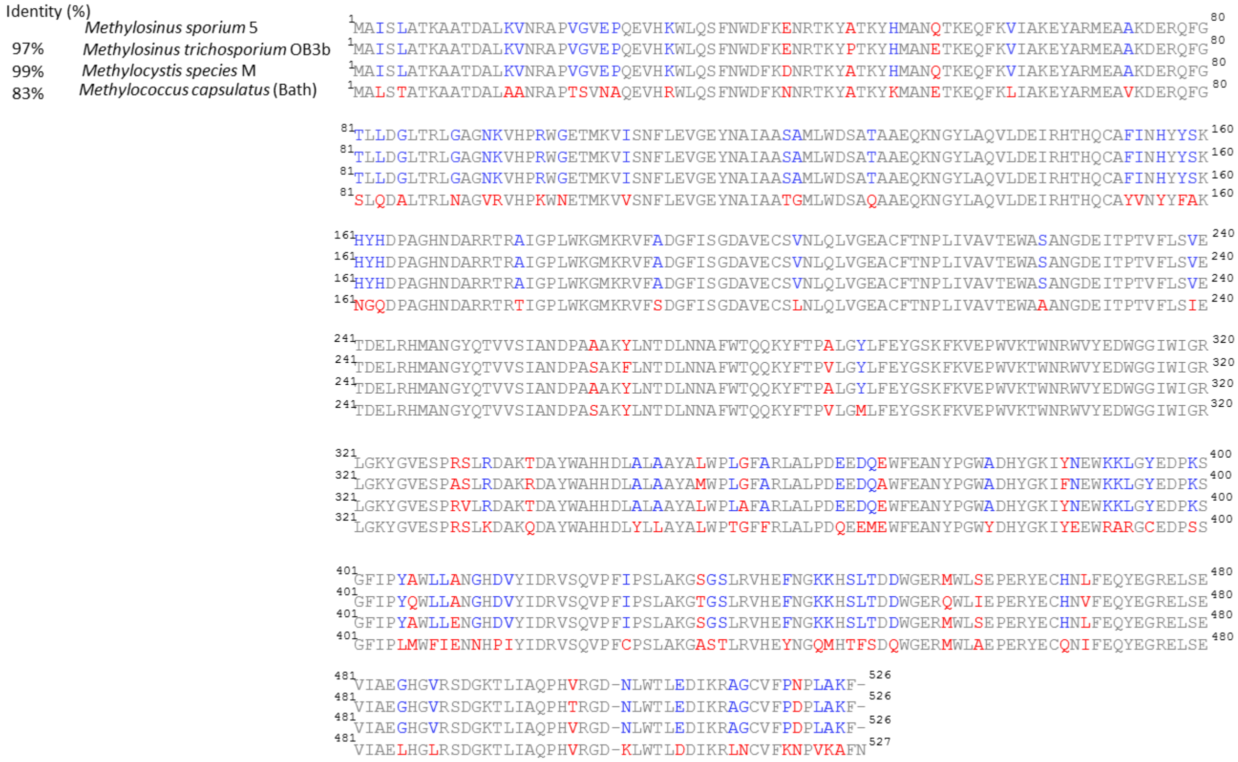
Keywords: Biocatalysts, Biocatalytic reaction, *Methylosinus sporium* strain 5, Soluble methane monooxygenase, C–H activation, O_2 activation

SUPPLEMENTARY FIGURES



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Identity (%)
        Methylosinus sporium 5                 1MAISLATKAATDALKVNRAPVGVEPQEVHKWLQSFNWDFKENRTKYATKYHMANQTKEQFKVIAKEYARMEAAKDERQFG 80
97%     Methylosinus trichosporium OB3b        1MAISLATKAATDALKVNRAPVGVEPQEVHKWLQSFNWDFKENRTKYPTKYHMANETKEQFKVIAKEYARMEAAKDERQFG 80
99%     Methylocystis species M                1MAISLATKAATDALKVNRAPVGVEPQEVHKWLQSFNWDFKDNRTKYATKYHMANQTKEQFKVIAKEYARMEAAKDERQFG 80
83%     Methylococcus capsulatus (Bath)        1MALSTATKAATDALAANRAPTSVNAQEVHRWLQSFNWDFKNNRTKYATKYKMANETKEQFKLIAKEYARMEAVKDERQFG 80

                                              81TLLDGLTRLGAGNKVHPRWGETMKVISNFLEVGEYNAIAASAMLWDSATAAEQKNGYLAQVLDEIRHTHQCAFINHYYSK 160
                                              81TLLDGLTRLGAGNKVHPRWGETMKVISNFLEVGEYNAIAASAMLWDSATAAEQKNGYLAQVLDEIRHTHQCAFINHYYSK 160
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                                              81SLQDALTRLNAGVRVHPKWNETMKVVSNFLEVGEYNAIAATGMLWDSAQAAEQKNGYLAQVLDEIRHTHQCAYVNYYFAK 160

                                             161HYHDPAGHNDARRTRAIGPLWKGMKRVFADGFISGDAVECSVNLQLVGEACFTNPLIVAVTEWASANGDEITPTVFLSVE 240
                                             161HYHDPAGHNDARRTRAIGPLWKGMKRVFADGFISGDAVECSVNLQLVGEACFTNPLIVAVTEWASANGDEITPTVFLSVE 240
                                             161HYHDPAGHNDARRTRAIGPLWKGMKRVFADGFISGDAVECSVNLQLVGEACFTNPLIVAVTEWASANGDEITPTVFLSVE 240
                                             161NGQDPAGHNDARRTRTIGPLWKGMKRVFSDGFISGDAVECSLNLQLVGEACFTNPLIVAVTEWAAANGDEITPTVFLSIE 240

                                             241TDELRHMANGYQTVVSIANDPAAAKYLNTDLNNAFWTQQKYFTPALGYLFEYGSKFKVEPWVKTWNRWVYEDWGGIWIGR 320
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                                             241TDELRHMANGYQTVVSIANDPAAAKYLNTDLNNAFWTQQKYFTPALGYLFEYGSKFKVEPWVKTWNRWVYEDWGGIWIGR 320
                                             241TDELRHMANGYQTVVSIANDPASAKYLNTDLNNAFWTQQKYFTPVLGMLFEYGSKFKVEPWVKTWNRWVYEDWGGIWIGR 320

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                                             401GFIPYQWLLANGHDVYIDRVSQVPFIPSLAKGTGSLRVHEFNGKKHSLTDDWGERQWLIEPERYECHNVFEQYEGRELSE 480
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                                             481VIAEGHGVRSDGKTLIAQPHVRGD-NLWTLEDIKRAGCVFPNPLAKF- 526
                                             481VIAEGHGVRSDGKTLIAQPHTRGD-NLWTLEDIKRAGCVFPDPLAKF- 526
                                             481VIAEGHGVRSDGKTLIAQPHVRGD-NLWTLEDIKRAGCVFPDPLAKF- 526
                                             481VIAELHGLRSDGKTLIAQPHVRGD-KLWTLDDIKRLNCVFKNPVKAFN 527
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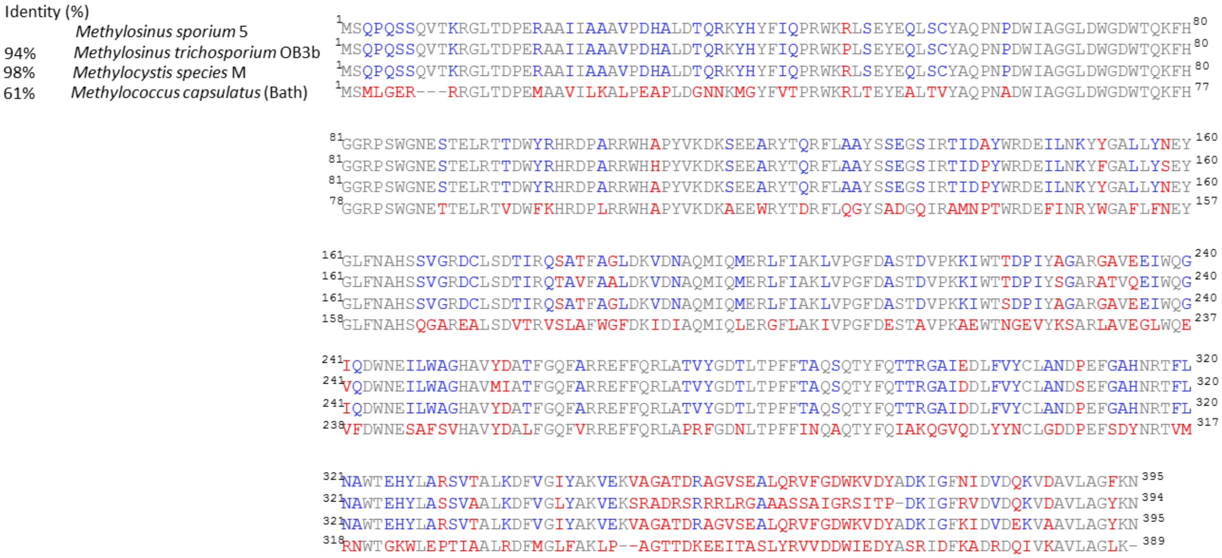
Figure S1. Multiple sequence amino acid alignment of sMMO from *Methylosinus sporium* 5 (type II), *Methylosinus trichosporium* OB3b (type II), *Methylocystis* species M (type II), and *Methylococcus capsulatus* Bath (type X).

(A) Alignment of amino acids from the α-subunit of MMOH (GenBank accession number: ABD46892). Gray and red colors represent identical and different amino acids, respectively. Blue represents the identical residues among type II methanotrophs.



Identity (%)
Methylosinus sporium 5
94% *Methylosinus trichosporium* OB3b
98% *Methylocystis species* M
61% *Methylococcus capsulatus* (Bath)

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1   MSQPQSSQVTKRGLTDPERAAIIAAAVPDHALDTQRKYHYFIQPRWKRLSEYEQLSCYAQPNPDWIAGGLDWGDWTQKFH 80
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1   MSQPQSSQVTKRGLTDPERAAIIAAAVPDHALDTQRKYHYFIQPRWKRLSEYEQLSCYAQPNPDWIAGGLDWGDWTQKFH 80
1   MSMLGER---RRGLTDPEMAAVILKALPEAPLDGNNKMGYFVTPRWKRLTEYEALTVYAQPNADWIAGGLDWGDWTQKFH 77

81  GGRPSWGNESTELRTTDWYRHRDPARRWHAPYVKDKSEEARYTQRFLAAYSSEGSIRTIDAYWRDEILNKYYGALLYNEY 160
81  GGRPSWGNESTELRTTDWYRHRDPARRWHHPYVKDKSEEARYTQRFLAAYSSEGSIRTIDPYWRDEILNKYFGALLYSEY 160
81  GGRPSWGNESTELRTTDWYRHRDPARRWHAPYVKDKSEEARYTQRFLAAYSSEGSIRTIDPYWRDEILNKYYGALLYNEY 160
78  GGRPSWGNETTELRTVDWFKHRDPLRRWHAPYVKDKAEEWRYTDRFLQGYSADGQIRAMNPTWRDEFINRYWGAFLFNEY 157

161 GLFNAHSSVGRDCLSDTIRQSATFAGLDKVDNAQMIQMERLFIAKLVPGFDASTDVPKKIWTTDPIYAGARGAVEEIWQG 240
161 GLFNAHSSVGRDCLSDTIRQTAVFAALDKVDNAQMIQMERLFIAKLVPGFDASTDVPKKIWTTDPIYSGARATVQEIWQG 240
161 GLFNAHSSVGRDCLSDTIRQSATFAGLDKVDNAQMIQMERLFIAKLVPGFDASTDVPKKIWTSDPIYAGARGAVEEIWQG 240
158 GLFNAHSQGAREALSDVTRVSLAFWGFDKIDIAQMIQLERGFLAKIVPGFDESTAVPKAEWTNGEVYKSARLAVEGLWQE 237

241 IQDWNEILWAGHAVYDATFGQFARREFFQRLATVYGDTLTPFFTAQSQTYFQTTRGAIEDLFVYCLANDPEFGAHNRTFL 320
241 VQDWNEILWAGHAVMIATFGQFARREFFQRLATVYGDTLTPFFTAQSQTYFQTTRGAIDDLFVYCLANDSEFGAHNRTFL 320
241 IQDWNEILWAGHAVYDATFGQFARREFFQRLATVYGDTLTPFFTAQSQTYFQTTRGAIDDLFVYCLANDPEFGAHNRTFL 320
238 VFDWNESAFSVHAVYDALFGQFVRREFFQRLAPRFGDNLTPFFINQAQTYFQIAKQGVQDLYYNCLGDDPEFSDYNRTVM 317

321 NAWTEHYLARSVTALKDFVGIYAKVEKVAGATDRAGVSEALQRVFGDWKVDYADKIGFNIDVDQKVDAVLAGFKN 395
321 NAWTEHYLASSVAALKDFVGLYAKVEKSRADRSRRRLRGAAASSAIGRSITP-DKIGFRVDVDQKVDAVLAGYKN 394
321 NAWTEHYLARSVTALKDFVGIYAKVEKVAGATDRAGVSEALQRVFGDWKVDYADKIGFKIDVDEKVAAVLAGYKN 395
318 RNWTGKWLEPTIAALRDFMGLFAKLP--AGTTDKEEITASLYRVVDDWIEDYASRIDFKADRDQIVKAVLAGLK- 389
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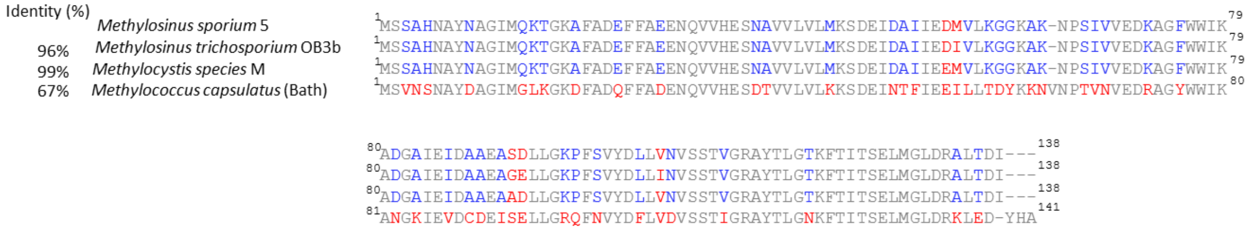
(B) Alignment of amino acids from the β-subunit of MMOH (GenBank accession number: ABD46893). Gray and red colors represent identical and different amino acids, respectively. Blue represents the identical residues among type II methanotrophs.

(C) Alignment of amino acids from the γ-subunit of MMOH (GenBank accession number: ABD46895). Gray and red colors represent identical and different amino acids, respectively. Blue represents the identical sequences among type II methanotrophs.



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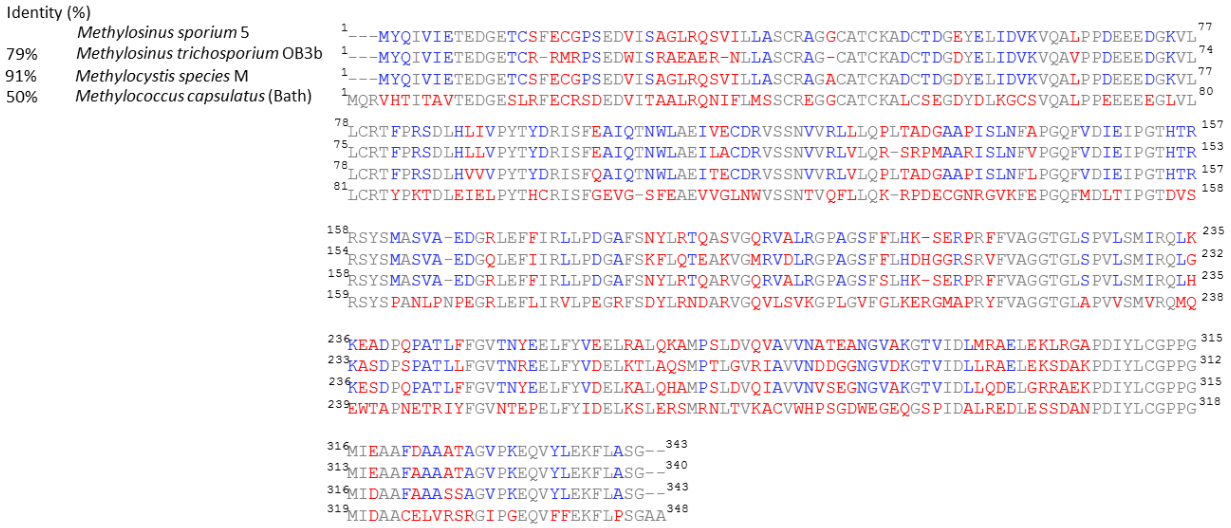
Identity (%)
     Methylosinus sporium 5               1MSSAHNAYNAGIMQKTGKAFADEFFAEENQVVHESNAVVLVLMKSDEIDAIIEDMVLKGGKAK-NPSIVVEDKAGFWWIK79
96%  Methylosinus trichosporium OB3b     1MSSAHNAYNAGIMQKTGKAFADEFFAEENQVVHESNAVVLVLMKSDEIDAIIEDIVLKGGKAK-NPSIVVEDKAGFWWIK79
99%  Methylocystis species M             1MSSAHNAYNAGIMQKTGKAFADEFFAEENQVVHESNAVVLVLMKSDEIDAIIEEMVLKGGKAK-NPSIVVEDKAGFWWIK79
67%  Methylococcus capsulatus (Bath)     1MSVNSNAYDAGIMGLKGKDFADQFFADENQVVHESDTVVLVLKKSDEINTFIEEILLTDYKKNVNPTVNVEDRAGYWWIK80

                                         80ADGAIEIDAAEASDLLGKPFSVYDLLVNVSSTVGRAYTLGTKFTITSELMGLDRALTDI---138
                                         80ADGAIEIDAAEAGELLGKPFSVYDLLINVSSTVGRAYTLGTKFTITSELMGLDRALTDI---138
                                         80ADGAIEIDAAEAADLLGKPFSVYDLLVNVSSTVGRAYTLGTKFTITSELMGLDRALTDI---138
                                         81ANGKIEVDCDEISELLGRQFNVYDFLVDVSSTIGRAYTLGNKFTITSELMGLDRKLED-YHA141
```

Figure S2. Multiple amino acid alignment of MMOB (GenBank accession: ABD46894) from *Methylosinus sporium* 5 (type II), *Methylosinus trichosporium* OB3b (type II), *Methylocystis species* M (type II), and *Methylococcus capsulatus* Bath (type X).



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Identity (%)
        Methylosinus sporium 5
79%     Methylosinus trichosporium OB3b
91%     Methylocystis species M
50%     Methylococcus capsulatus (Bath)

1   ---MYQIVIETEDGETCSFECGPSEDVISAGLRQSVILLASCRAGGCATCKADCTDGEYELIDVKVQALPPDEEEDGKVL 77
1   ---MYQIVIETEDGETCR-RMRPSEDWISRAEAER-NLLASCRAG-CATCKADCTDGDYELIDVKVQAVPPDEEEDGKVL 74
1   ---MYQIVIETEDGETCSFECGPSEDVISAGLRQSVILLASCRAGACATCKADCTDGDYELIDVKVQALPPDEEEDGKVL 77
1   MQRVHTITAVTEDGESLRFECRSDEDVITAALRQNIFLMSSCREGGCATCKALCSEGDYDLKGCSVQALPPEEEEEGLVL 80

78  LCRTFPRSDLHLIVPYTYDRISFEAIQTNWLAEIVECDRVSSNVVRLLLQPLTADGAAPISLNFAPGQFVDIEIPGTHTR 157
75  LCRTFPRSDLHLLVPYTYDRISFEAIQTNWLAEILACDRVSSNVVRLVLQR-SRPMAARISLNFVPGQFVDIEIPGTHTR 153
78  LCRTFPRSDLHVVVPYTYDRISFQAIQTNWLAEITECDRVSSNVVRLVLQPLTADGAAPISLNFLPGQFVDIEIPGTHTR 157
81  LCRTYPKTDLEIELPYTHCRISFGEVG-SFEAEVVGLNWVSSNTVQFLLQK-RPDECGNRGVKFEPGQFMDLTIPGTDVS 158

158 RSYSMASVA-EDGRLEFFIRLLPDGAFSNYLRTQASVGQRVALRGPAGSFFLHK-SERPRFFVAGGTGLSPVLSMIRQLK 235
154 RSYSMASVA-EDGQLEFIIRLLPDGAFSKFLQTEAKVGMRVDLRGPAGSFFLHDHGGRSRVFVAGGTGLSPVLSMIRQLG 232
158 RSYSMASVA-EDGRLEFFIRLLPDGAFSNYLRTQARVGQRVALRGPAGSFSLHK-SERPRFFVAGGTGLSPVLSMIRQLH 235
159 RSYSPANLPNPEGRLEFLIRVLPEGRFSDYLRNDARVGQVLSVKGPLGVFGLKERGMAPRYFVAGGTGLAPVVSMVRQMQ 238

236 KEADPQPATLFFGVTNYEELFYVEELRALQKAMPSLDVQVAVVNATEANGVAKGTVIDLMRAELEKLRGAPDIYLCGPPG 315
233 KASDPSPATLLFGVTNREELFYVDELKTLAQSMPTLGVRIAVVNDDGGNGVDKGTVIDLLRAELEKSDAKPDIYLCGPPG 312
236 KESDPQPATLFFGVTNYEELFYVDELKALQHAMPSLDVQIAVVNVSEGNGVAKGTVIDLLQDELGRRAEKPDIYLCGPPG 315
239 EWTAPNETRIYFGVNTEPELFYIDELKSLERSMRNLTVKACVWHPSGDWEGEQGSPIDALREDLESSDANPDIYLCGPPG 318

316 MIEAAFDAAATAGVPKEQVYLEKFLASG-- 343
313 MIEAAFAAAATAGVPKEQVYLEKFLASG-- 340
316 MIDAAFAAASSAGVPKEQVYLEKFLASG-- 343
319 MIDAACELVRSRGIPGEQVFFEKFLPSGAA 348
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Figure S3. Multiple amino acid alignment of MMOR (GenBank accession: ABD46897) from *Methylosinus sporium* 5 (type II), *Methylosinus trichosporium* OB3b (type II), *Methylocystis species* M (type II), and *Methylococcus capsulatus* Bath (type X).

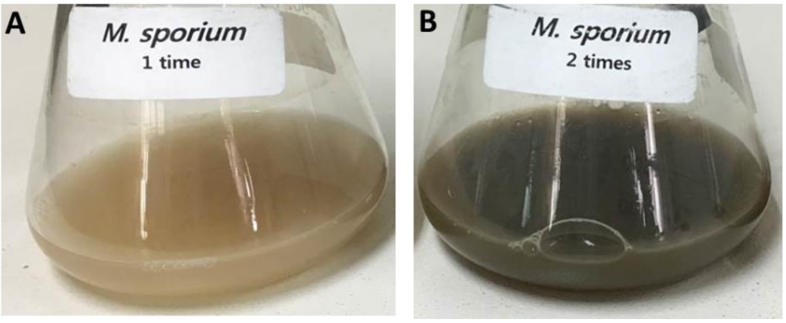

Figure S4. Growth and pigmentation profile of sMMO from *M. sporium* 5 based on optical density at 600 nm (OD_{600}). (A) Cell growth at OD_{600} of 3.0 with one-time methane feeding (once/day). (B) Cell growth at OD_{600} of 8.2 with two-time methane supply (twice/day). Cultures A and B were proliferated for a week in media with the same iron concentration (20.4 mg/mL).

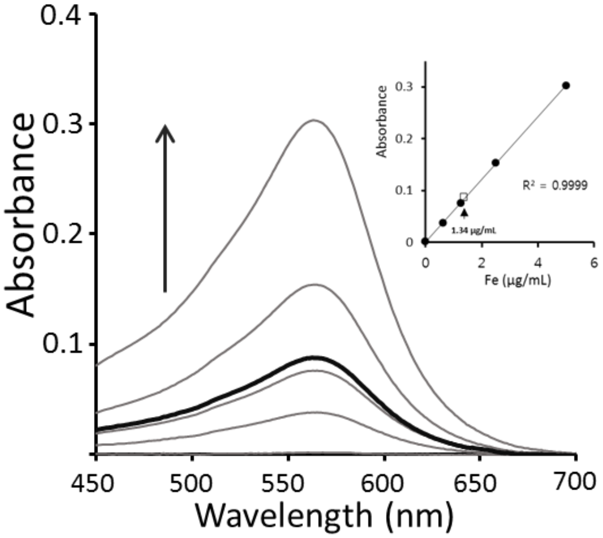

Figure S5. Ferrozine assay of MMOH showed iron contents in MMOH (3.8–4.1 Fe/MMOH). The maximum wavelength was monitored at 562 nm from the iron–ferrozine complex. Heavy and light lines represent denatured MMOH and standard solutions, respectively. All experiments were performed in triplicate with R^2 values > 0.999.

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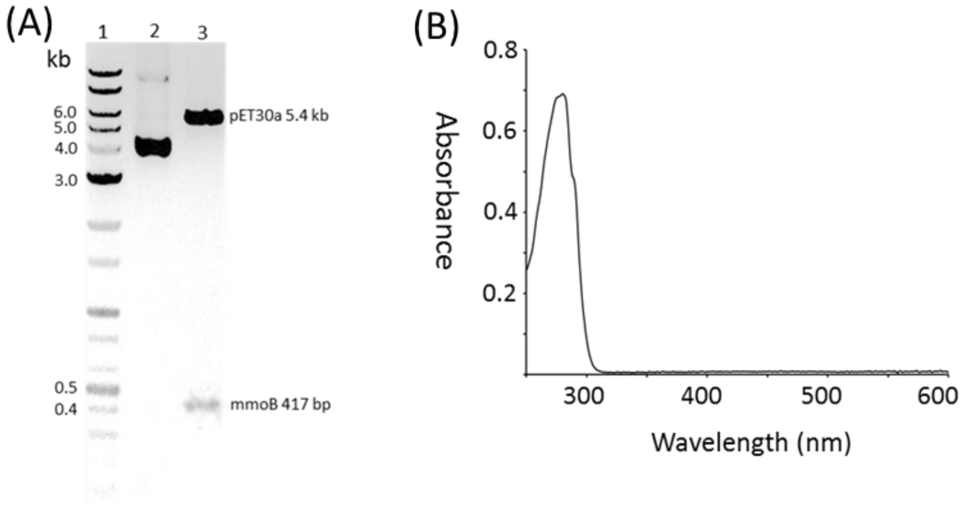

Figure S6. Constructed pET30a-*mmoB* plasmids and UV-visible spectrum of MMOB after purification. (A) Lane 1 represents the control ladder, lane 2 represents the pET30a-*mmoB* construct, and lane 3 represents nucleotides after *Nde*I and *Hind*III double digestion. (B) UV-vis spectrum of MMOB after construction.

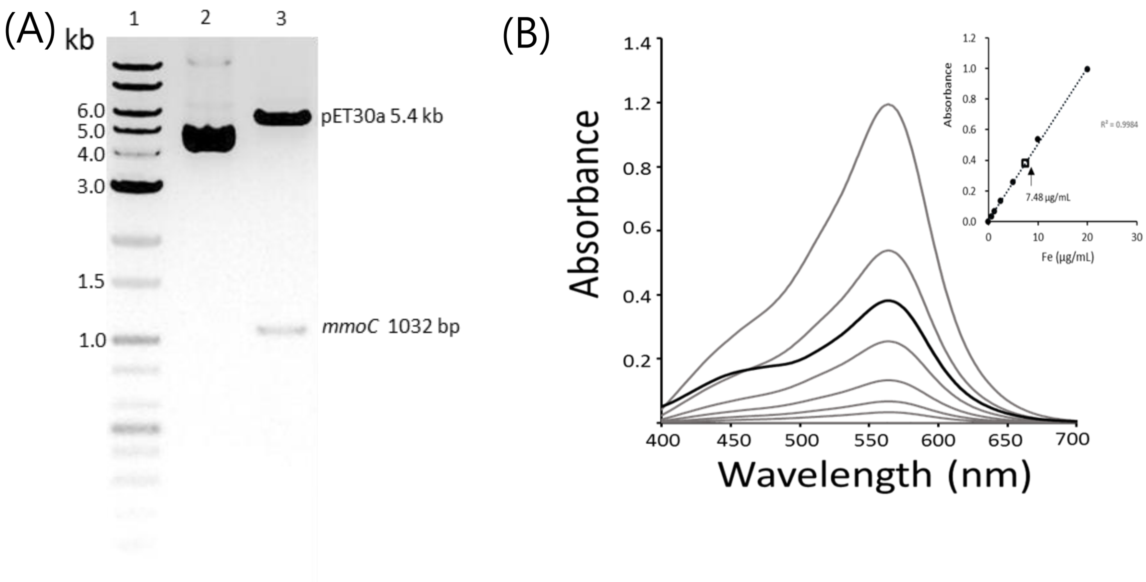

Figure S7. Constructed *pET30a-mmoC* plasmid and ferrozine assays from MMOR.

(A) Lane 1 represents the control ladder, lane 2 represents the *pET30a-mmoC* construct, and lane 3 represents the *pET30a-mmoC* after *Nde*I and *Hind*III double digestion. (B) Ferrozine assay of MMOR for demonstrating the iron content in MMOR (2.03–2.14 Fe/MMOR). The maximum wavelength was monitored at 562 nm from the iron–ferrozine complex. Heavy and light lines represent iron solutions from denatured MMOR and standard solutions, respectively. All experiments were performed in triplicate with R^2 values > 0.998.

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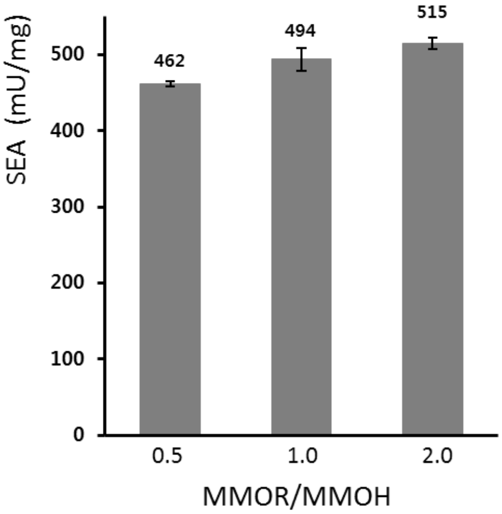

Figure S8. Specific enzyme activity based on the ratio of MMOR to MMOH in 2 mol equivalents of MMOB at pH 7.5.

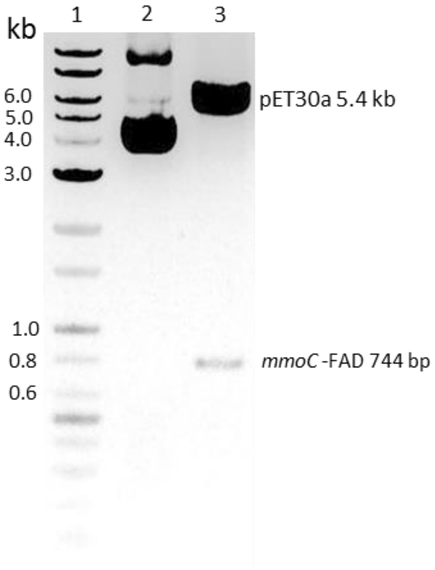

Figure S9. Constructed *pET30a-mmoC-FAD* plasmids. Lane 1 represents the control ladder, lane 2 represents the *pET30a-mmoC* construct, and lane 3 represents nucleotides after *Nde*I and *Hind*III double digestion.

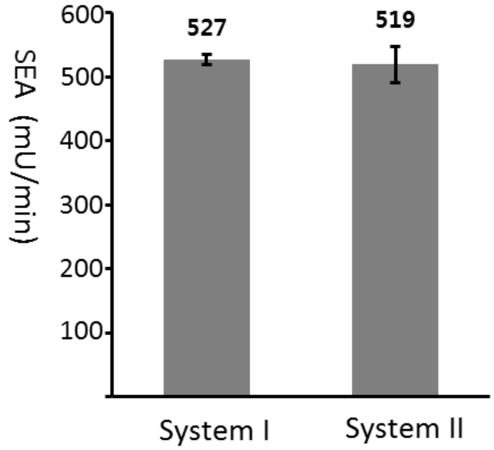

Figure S10. Measurement of crossreactivity in MMOB and MMOR. System I consists of MMOH (*M. sporium* 5), MMOB (*M. species* M), and MMOR (*M. sporium* 5). System II consists of MMOH (*M. sporium* 5), MMOB (*M. sporium* 5), and MMOR (*M. species* M). Specific enzymatic activities were measured at 30°C and pH 7.5.

SUPPLEMENTARY TABLE

Table S1. Primers used in this study.

Primer name	Sequence (5′-3′)	Enzymes (strains)
mmoB-M-Fd	aacatatgtctagcgcgc	MMOB (*M. sporium* 5)
mmoB-M-Rv	aagcttcaaatatcggtcaggg	MMOB (*M. sporium* 5)
mmoB-MM-Fd	aacatatgtctagcgcgc	MMOB (*M. species* M)
mmoB-MM-Rv	gctcagctcaaatgtcggtcag	MMOB (*M. species* M)
mmoC-M-Fd	gcgcatatgtaccagatcgt	MMOR (*M. sporium* 5)
mmoC-M-Rv	ataagcttcagccgctcgc	MMOR (*M. sporium* 5)
mmoC-MM-Fc	gcggcatatgtatcagatcgtc	MMOR (*M. species* M)
mmoC-MM-Rv	atatagctcagccgctcgccag	MMOR (*M. species* M)
mmoC-FAD-M-Fd	agaccatatgcgcatctccttcga	FAD domain (*M. sporium* 5)
mmoC-FAD-M-Rv	tataagcttcagccgctcgcca	FAD domain (*M. sporium* 5)