Extremophiles (2012) 16:405–409
DOI 10.1007/s00792-012-0439-y

ORIGINAL PAPER

Differential expression of particulate methane monooxygenase genes in the verrucomicrobial methanotroph 'Methylacidiphilum kamchatkense' Kam1

Helge-André Erikstad · Sigmund Jensen · T. Jeffrey Keen · Nils-Kåre Birkeland

Received: 8 December 2011 / Accepted: 22 March 2012 / Published online: 10 April 2012

Abstract Methane monooxygenases (MMOs) are oxygen-dependent enzymes that catalyze the oxidation of methane to methanol in the methanotrophic bacteria. The thermoacidophilic verrucomicrobial methanotroph 'Methylacidiphilum kamchatkense' Kam1 contains three complete and phylogenetically distinct copies of the *pmoCAB* gene cluster apparently organized as operons, each encoding all three subunits of particulate MMO (pMMO), and a truncated *pmoCA* cluster encoding only two of the subunits. Two of the clusters are present as a tandem array, but the other clusters occur in isolation. Here, the expression of these clusters has been assessed using the four *pmoA* genes as targets in reverse transcriptase quantitative PCR analysis. One of the *pmoA* genes, designated *pmoA2*, is at least 35-fold more strongly transcribed than the other *pmoA* copies. Growth at suboptimal temperature and pH conditions did not significantly change the transcription pattern, indicating that the *pmoCAB2* cluster encodes the functional pMMO under methane-fuelled growth conditions. During growth on methanol, expression of *pmoA2* was reduced approximately tenfold as compared to growth on methane, suggesting a role for the alternative carbon substrates in gene regulation.

Keywords *Verrucomicrobia* · Methanotroph · *pmo* · mRNA · q-PCR · Thermoacidophile

Communicated by A. Driessen.

H.-A. Erikstad · S. Jensen · T. J. Keen · N.-K. Birkeland (✉)
Department of Biology, University of Bergen,
P.O. Box 7800, 5020 Bergen, Norway
e-mail: nils.birkeland@bio.uib.no

S. Jensen · T. J. Keen · N.-K. Birkeland
Centre for Geobiology, University of Bergen,
P.O. Box 7800, 5020 Bergen, Norway

Introduction

Biological methane oxidation carried out by aerobic bacteria is an important component of the global carbon cycle that reduces the atmospheric release of this major greenhouse gas. There are now 18 characterized genera of these ubiquitous but metabolically specialized bacteria (Murrell et al. 2008; Geymonat et al. 2011). In addition, an anaerobic denitrifying methanotroph that can couple methane oxidation to nitrite reduction via an 'intra-aerobic' pathway has been identified (Ettwig et al. 2010). In contrast to the consortia of microorganisms that carry out anaerobic methane oxidation linked to sulphate reduction (reviewed in Colwell et al. 2008), aerobic methanotrophs can grow independently.

The key enzyme in methanotrophs, methane monooxygenase (MMO), is found in two forms; the particulate MMO (pMMO) is associated with intracellular membranes whereas the soluble MMO (sMMO) is free in the cytoplasm. These enzymes oxidize methane to methanol, thereby initiating the aerobic pathway for methane assimilation (Bedard and Knowles 1989; Hanson and Hanson 1996). The pMMO has been found in all described methanotrophs except for strains of the moderately acidophilic genera, *Methylocella* and *Methyloferula*, that are found in peat bog environments (Dedysh et al. 2005; Vorobev et al. 2011). *Methylocella* relies solely on sMMO for methane oxidation, but unlike other methanotrophs *Methylocella* spp. are facultative methanotrophs and are able to grow on simple multi-carbon compounds that actually repress the sMMO activity (Dedysh et al. 2005). Other sMMO-containing methanotrophs down-regulate their sMMO activity when grown at high copper-to-biomass ratios, allowing methane oxidation to proceed by the more efficient copper-dependent pMMO, a mechanism mediated by

a transcriptional switch between *mmo* (which encodes sMMO) and *pmo* gene expression (Nielsen et al. 1996; Murrell et al. 2000). In contrast to the sMMO, which appears to be encoded by a single operon, methanotrophs often possess several *pmo* operons. The pMMO consists of three subunits encoded by the *pmoCAB* gene cluster. Two complete nearly identical *pmoCAB* operons have been found in *Methylosinus trichosporium* OB3b, *Methylocysits* sp. strain M. (Gilbert et al. 2000), and *M. capsulatus* Bath (Semrau et al. 1995; Stolyar et al. 1999). In *Methylocystis* sp. strain SC2, a third *pmo* cluster has been found (termed *pmoCAB2*) which differs significantly in sequence similarity to the other two operons (termed *pmoCAB1*) (Yimga et al. 2003; Ricke et al. 2004). The *pmoCAB2* operon encodes a novel type of pMMO (termed pMMO2) with a significantly higher affinity for methane than any enzymes studied previously (Baani and Liesack 2008), enabling the SC2 strain to oxidize methane at atmospheric concentrations. Expression of *pmoCAB2* was shown to be constitutive, while the *pmoCAB1* genes were down-regulated under low methane mixing ratios (Baani and Liesack 2008). The molecular mechanism for the regulation of the *pmoCAB* operons in methanotrophs as a response to copper and methane has not yet been described in detail.

The thermoacidophilic Verrucomicrobial methanotrophs isolated from terrestrial geothermal areas possess three diverse but phylogenetically distinct *pmoCAB* operons (Dunfield et al. 2007; Pol et al. 2007; Op den Camp et al. 2009), suggesting a well-developed and flexible methane uptake machinery in these bacteria. The ratios of non-synonymous versus synonymous substitution rates for the three *pmoA* orthologues are very low and thus appear to be under strong purifying selection pressure, indicating that each play functionally distinct roles (Op den Camp et al. 2009). Here, we have analyzed the expression of four different *pmoA* copies in the verrucomicrobial methanotroph, 'Methylacidiphilum kamchatkense' strain Kam1 (Islam et al. 2008), as well as the phylogenetic relationships of its pMMO proteins.

Materials and methods

Cultivation and harvesting of 'M. kamchatkense'

'M. kamchatkense' Kam1 was grown in a 1:10 dilution of a standard methanotrophic mineral medium (Whittenbury et al. 1970; Dedysh et al. 1998) adjusted to pH 3.5 using 1 M HCl. Cultivation was carried out in sealed 120 ml serum bottles containing 10 ml medium as previously described (Islam et al. 2008). Methane or methanol was added by a syringe to 30 % final headspace concentration or 0.1 % in the liquid phase, respectively. Cultures inoculated with Kam1 were incubated at 55 °C with shaking at 150 rpm. Growth was monitored using a counting chamber. The cells were harvested at densities of $\sim 1 \times 10^9$ cells ml^{-1} by centrifugation at $3500 \times g$ for 30 min at 4 °C. Prior to the centrifugation the cells were cooled in an ethanol/dry-ice bath and the cell pellet was resuspended in a RNA later solution (Ambion). Following a 5 min incubation at room temperature and centrifugation the cell pellet was frozen at −80 °C.

RNA extraction

Total RNA was extracted from thawed cell pellets using an RNeasy plus mini kit (Qiagen), with slight modifications. Briefly, the pellet was resuspended in 200 µl of a TE lysis buffer (pH 8.0) containing lysozyme (15 mg ml^{-1}) and proteinase K (20 µg ml^{-1}). Following 10 s vortexing and incubation for 10 min at room temperature with shaking every 2 min, 600 µl RLT buffer with β-mercaptoethanol (10 µl ml^{-1}) was added. The lysate was transferred to the gDNA spin column and the extraction continued according to the manufacturer's protocol (steps 4–7). To improve removal of DNA the lysate on the gDNA column membrane was incubated for 15 min at room temperature with a mixture of the RDD buffer (70 µl) supplemented with DNaseI (10 µl). Following addition of 350 µl RW1 buffer and 5 min incubation at room temperature, the gDNA column was centrifuged at $\geq 8000 \times g$ for 15 s. Further preparation of the RNA extract was performed according to Qiagen's protocol. RNA content and quality was evaluated using agarose gel electrophoresis and the Nanodrop (Thermo Scientific ND-1000).

Quantitative real-time PCR

Four individual forward primers and a common reverse primer were designed to amplify Kam1 pmoA transcripts. The primers were designated pmoA1f (5′-CATCAGATGCTATTGGCTGG-3′), pmoA2f (5′-CATCAGATGCTGACAGCCGG-3′, pmoA3f (5′-AAGGAGACTGGAGCTTCTGG-3′), pmoA4f (5′-CAACTCTTTGCTGGAGACTGG-3′), pmoA1r/pmoA2r (5′-CCTRTTCATCCACTGRCCAA-3′), pmoA3r (5′-CCACTGCCAGAGATTGATTCC-3′) and pmoA4r (5′-CCGATTCAACCATTGTCCAAG-3′). Homology searches showed that these primers were specific for verrucomicrobial methanotrophs. Primer specificity was checked by PCR using cDNA as template followed by sequencing of the amplicons. The sequences of the single PCR products obtained for all four primer pairs matched exactly the sequence of the four *pmoA* target genes. Total RNA was added to the master mix in 0.2 ml tubes, each containing 200 nM of the respective forward and reverse primers. Express Sybr GreenER qPCR and Two-Step qRT-PCR kits (Invitrogen) were used. The

cDNA synthesis was carried out in a total volume of 20 µl following the manufacturer's instructions. The tubes were incubated in an Opticom DNA Engine 2 at 25 °C for 10 min, 42 °C for 60 min followed by the termination of the reaction at 85 °C for 5 min. Cycling was performed using an Opticom DNA Engine 2 with 2 min at 50 °C and 2 min at 95 °C followed by 40 cycles of 15 s at 95 °C, 1 min at 60 °C, 15 s at 72 °C, 1 s at 83 °C (plate read), and thereafter 68 °C for 10 min, 1 s per 0.5 °C from 60 to 95 °C (melting curve), and finally 5 min at 68 °C. The standard curve used in the experiments contained tenfold dilutions of genomic Kam1 DNA ranging from ~80 to 0.00008 ng μl^{-1}. Three parallel experiments were performed from three separate cultures.

Results

The *pmo* operons

A draft genome sequence of 'M. kamchatkense' Kam1 has previously been generated, consisting of ~2.4 Mb present as 1043 contigs with an N50 size of 172155 nt (Op den Camp et al. 2009). The average nucleotide sequence identity between Kam1 and the fully sequenced strain V4 (Hou et al. 2008) is approximately 72 %. Four *pmo* operons were identified in Kam1, the highest number currently known for any methanotroph. No genes encoding a sMMO were detected. Operons *pmoCAB1* and *pmoCAB2* are located in a tandem array separated by 500 nt of intergenic DNA (Fig. 1). Operon *pmo4* is incomplete, lacking the *B* gene. A solitary *C5* gene occur 1.6 kb downstream from *pmoB2* (Fig. 1).

A phylogenetic tree based on concatenated sequences of all the translated verrucomicrobial *pmoCAB* gene clusters, including corresponding concatemers from representative proteobacterial methanotrophs was constructed (Fig. 2). Except for a relatively weak affiliation of the strain V4 PmoCAB1 sequences with those of strains Kam1 and SolV, this tree confirms the distinct phylogenetic grouping of all three verrucomicrobial MMO subunits into three clades, as previously shown for the PmoA sequences (Op den Camp et al. 2009), and further supports the notion of a possible diversification of verrucomicrobial pMMOs into three functional groups. The non-synonymous versus synonymous base substitution rate ratios (d*N*/d*S* or ω) for the three orthologous *pmoA* genes have previously been shown to be very low, indicating that each of the genes are functional, and also under a strong purifying selection pressure (Op den Camp et al. 2009). Very low ω values were estimated also for the *pmo*C and *pmo*B genes (Fig. 2) using the MEGA5 evolutionary analyses package (Tamura et al. 2011), thus showing that the entire three gene clusters are subject to strong purifying selection pressure.

Expression of *pmoA*

To determine the expression pattern of the *pmo* operons in Kam1 a quantitative real-time PCR analysis of the *pmoA* genes was performed using primers specifically targeting the mRNA of each of the four genes. The results indicated large differences in expression levels during growth on methane, with *pmoA2* being the most highly expressed (Fig. 3). The *pmo1* operon, located upstream of *pmo2*, was expressed at a much lower level than *pmoA2* (~1000-fold lower) (Fig. 3). *pmoA3* was expressed at an intermediate level, but still about 35-fold lower than *pmoA2*. These results indicate that *pmo2* encodes the functional pMMO enzyme under these growth conditions. However, growth at suboptimal pH values (pH 2.1 and 4.9) and at a suboptimal temperature (37 °C) only marginally affected the *pmoA2* expression or the relative abundance of mRNA from all four *pmoA* genes (results not shown). A tenfold down-regulation of *pmoA2* was, however, observed when Kam1 was grown on methanol as carbon and energy source (Fig. 3), demonstrating that *pmo2* is subject to a regulatory response possibly mediated by the presence or absence of methane. The expression of the other three *pmoA* genes was also affected by the substrate switch from methane to methanol, but to a lesser extent than *pmoA2* (Fig. 3). A putative transcription termination signal consisting of a hairpin sequence was identified immediately after the *pmoB1* gene, further implying independent regulation of the *pmo1* and *pmo2* gene clusters.

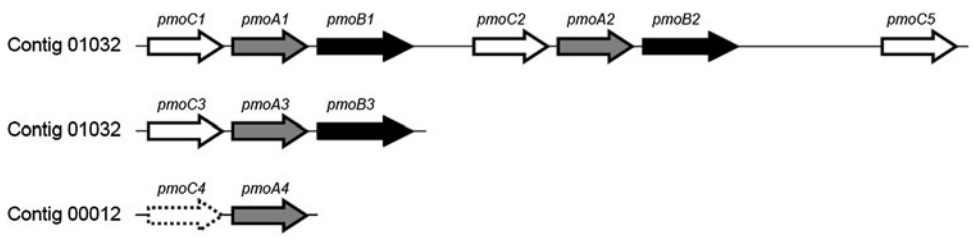

Fig. 1 Organization of *pmo* genes in the *Methylacidiphilum kamchatkense* Kam1 genome. There are three complete *pmoCAB* operons (1, 2, and 3). Operons 1 and 2 occur in tandem and are separated by ca. 0.5 kb, while operon 3 is ca. 100 kb distant to 1 and 2. A single *C5* gene is located ca. 1.6 kb downstream of *pmoCAB2*. The incomplete *pmoCA4* operon is unconnected to the operons 1, 2 and 3 and harbour an unusual *C4* gene. Accession numbers; *pmo* operons 1 and 2, JQ034363; *pmo* operon 3, JQ034364; *pmo4*, FJ462791

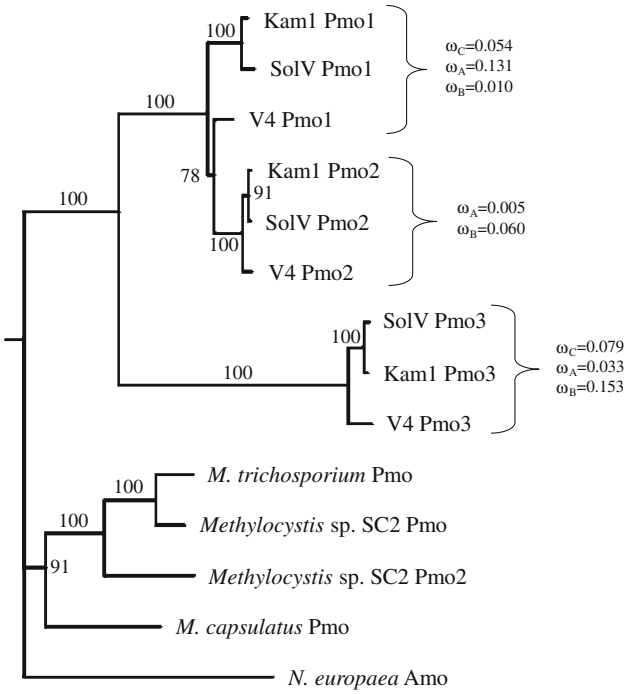

Fig. 2 Phylogenetic tree constructed based on concatenated amino acid sequences of the PmoC, A, B proteins of the three methanotrophic Verrucomicrobia isolates, 'Methylacidiphilum kamchatkense' (Kam1), 'Methylacidiphilum fumariolicum' (SolV) and 'Methylacidiphilum infernorum' (V4), and corresponding amino acid sequences of selected proteobacterial methanotrophs. Accession numbers; Kam1 *pmo* operons 1 and 2, JQ034363; Kam1 *pmo* operon 3, JQ034364; V4, CP000975; SolV *pmo* operon 1, EF591085; SolV *pmo* operon 2, EF591086; SolV *pmo* operon 3, EF591087; *M. trichosporium*, U31650; *Methylocystis* sp. SC2 *pmo*, AJ584611; *Methylocystis* sp. SC2 *pmo2*, BX649604; *M. capsulatus*, NC_002977; *N. europaea*, NP_841016, NP_841017 and NP_841018. The tree was constructed using the neighbour-joining algorithm as implemented in the PHYLIP version 3.65 phylogeny package with the ammonium monooxygenase (Amo) CAB concatemer sequence from *N. europaea* as outgroup. The bootstrap values (in %) are indicated at the nodes. The ratio of non-synonymous versus synonymous nucleotide base substitution rates for the *pmo*C, A and B genes (indicated with ω values) was calculated separately for the three verrucomicrobial *pmo* clusters using MEGA version 5 (Tamura et al. 2011)

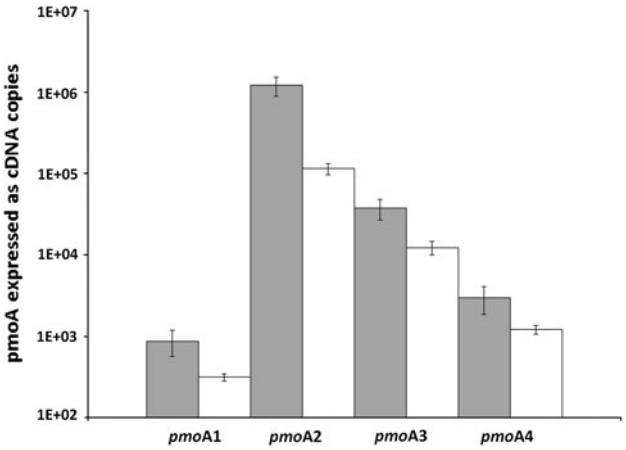

Fig. 3 Level of *pmo*A gene expression in *M. kamchatkense* growing optimally at pH 3.5 and 55 °C with methane (*grey*) or methanol (*white*) as the carbon and energy source. The number of *pmoA* transcripts quantified are given per 5 ng total RNA. *Error bars* indicate standard deviations ($n = 3$)

The standard medium that was used for cultivation of strain Kam1 contained 0.08 μM of copper. Increasing the copper concentration to 0.8 μM or reducing it to 0.008 μM appeared to inhibit growth which is in contrast to proteobacterial methanotrophs, in which efficient expression of the *pmo* operons depends on copper concentrations above 2–4 μM (for a recent review see Semrau et al. 2010).

Discussion

We were able to detect mRNA from all of the four *pmoA* genes of 'M. kamchatkense' Kam1 in this first study of verrucomicrobial *pmo* gene expression, demonstrating that they all are being expressed. These findings are in concordance with the conserved and distinct phylogenetic clustering and low nucleotide substitution rates observed between the *pmoCAB* genes, implying that they encode important biological functions and are under intense selective pressure. Although Kam1 is equipped with an extra *pmo* operon, the overall genomic arrangement of the other operons is similar to that found in strain V4, including the location of an orphan *pmoC* gene. An approximately tenfold down-regulation of *pmoA2* expression occurred when the carbon and energy source was shifted from methane to methanol. Individual *pmoA* gene expression has to our knowledge only been investigated for the two *pmoA* genes present in *Methylococcus capsulatus* Bath (Stolyar et al. 2001), *Methylocystis* strain SC2 (Baani and Liesack 2008) and in *Crenothrix polyspora* (Stoecker et al. 2006). Experiments with *M. capsulatus* Bath showed that pMMO is regulated as response to copper ions, increasing with increasing copper concentrations and transcribed from a σ70-like promoter (Stolyar et al. 2001). In *C. polyspora*, transcription of pMMO has been shown to be positively regulated by substrate availability (Stoecker et al. 2006). In *Methylocystis* SC2 two different pMMO enzymes are present, one (pMMO1) which is the usual variant seen in most methanotrophs, and a pMMO2, which is unusual in terms of its methane oxidation kinetics. While pMMO1, encoded by the inducible operon *pmo*CAB1, oxidized methane at concentrations >600 ppmv, but was shut down at lower concentrations, the isoenzyme pMMO2, encoded by the constitutively expressed *pmo*CAB2 operon, oxidized methane also at atmospheric 1.75 ppmv concentrations (Baani and Liesack 2008). It is possible that similar mechanisms exist in Kam1 that fine

tune the specific pMMO expressed to the availability of methane. Additional studies will be required to demonstrate whether this is true under alternative growth conditions.

Acknowledgments This work was supported by the Research Council of Norway (grant 204797), Statoil (grant 4501359564), the Norwegian Academy of Science and Statoil program (VISTA) (grant 6504), and Centre for Geobiology, University of Bergen (grant 802001). The excellent technical support of Staff Engineer Marit Steine Madsen is highly appreciated.

References

Baani M, Liesack W (2008) Two isozymes of particulate methane monooxygenase with different methane oxidation kinetics are found in *Methylocystis* sp strain SC2. Proc Natl Acad Sci USA 105:10203–10208

Bedard C, Knowles R (1989) Physiology, biochemistry, and specific inhibitors of CH_4, NH_4^+, and CO oxidation by methanotrophs and nitrifiers. Microbiol Rev 53:68–84

Colwell FS, Caldwell SL, Laidler JR, Brewer EA, Eberly JO, Sandborgh SC (2008) Anaerobic oxidation of methane: mechanisms, bioenergetics, and the ecology of associated microorganisms. Environ Sci Technol 42:6791–6799

Dedysh SN, Panikov NS, Tiedje JM (1998) Acidophilic methanotrophic communities from *Sphagnum* peat bogs. Appl Environ Microbiol 64:922–929

Dedysh SN, Knief C, Dunfield PF (2005) *Methylocella* species are facultatively methanotrophic. J Bacteriol 187:4665–4670

Dunfield PF, Yuryev A, Senin P, Smirnova AV, Stott MB, Hou SB, Ly B, Saw JH, Zhou ZM, Ren Y, Wang JM, Mountain BW, Crowe MA, Weatherby TM, Bodelier PLE, Liesack W, Feng L, Wang L, Alam M (2007) Methane oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia. Nature 450:879–882

Ettwig KF, Butler MK, Le Paslier D, Pelletier E, Mangenot S, Kuypers MMM, Schreiber F, Dutilh BE, Zedelius J, de Beer D, Gloerich J, Wessels HJCT, van Alen T, Luesken F, Wu ML, van de Pas-Schoonen KT, Op den Camp HJM, Janssen-Megens EM, Francoijs KJ, Stunnenberg H, Weissenbach J, Jetten MSM, Strous M (2010) Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. Nature 464:543–548

Geymonat E, Ferrando L, Tarlera SE (2011) *Methylogaea oryzae* gen. nov., sp nov., a mesophilic methanotroph isolated from a rice paddy field. Int J Syst Evol Microbiol 61:2568–2572

Gilbert B, McDonald IR, Finch R, Stafford GP, Nielsen AK, Murrell JC (2000) Molecular analysis of the pmo (particulate methane monooxygenase) operons from two type II methanotrophs. Appl Environ Microbiol 66:966–975

Hanson RS, Hanson TE (1996) Methanotrophic bacteria. Microbiol Rev 60:439–471

Hou S, Makarova KS, Saw JH, Senin P, Ly BV, Zhou Z, Ren Y, Wang J, Galperin MY, Omelchenko MV, Wolf YI, Yutin N, Koonin EV, Stott MB, Mountain BW, Crowe MA, Smirnova AV, Dunfield PF, Feng L, Wang L, Alam M (2008) Complete genome sequence of the extremely acidophilic methanotroph isolate V4, *Methylacidiphilum infernorum*, a representative of the bacterial phylum *Verrucomicrobia*. Biol Direct 3:1–25

Islam T, Jensen S, Reigstad LJ, Larsen Ø, Birkeland NK (2008) Methane oxidation at 55 °C and pH 2 by a thermoacidophilic bacterium belonging to the *Verrucomicrobia* phylum. Proc Natl Acad Sci USA 105:300–304

Murrell JC, McDonald IR, Gilbert B (2000) Regulation of expression of methane monooxygenases by copper ions. Trends Microbiol 8:221–225

Murrell JC, McDonald IR, Bodrossy L, Chen Y (2008) Molecular ecology techniques for the study of aerobic methanotrophs. Appl Environ Microbiol 74:1305–1315

Nielsen AK, Gerdes K, Degn H, Murrell JC (1996) Regulation of bacterial methane oxidation: transcription of the soluble methane monooxygenase operon of *Methylococcus capsulatus* (Bath) is repressed by copper ions. Microbiology 142:1289–1296

Op den Camp HJM, Islam T, Stott MB, Harhangi HR, Hynes A, Schouten S, Jetten MSM, Birkeland NK, Pol A, Dunfield PF (2009) Environmental, genomic and taxonomic perspectives on methanotrophic *Verrucomicrobia*. Environ Microbiol Rep 1:293–306

Pol A, Heijmans K, Harhangi HR, Tedesco D, Jetten MS, Op den Camp HJM (2007) Methanotrophy below pH 1 by a new Verrucomicrobia species. Nature 450:874–878

Ricke P, Erkel C, Kube M, Reinhardt R, Liesack W (2004) Comparative analysis of the conventional and novel pmo (Particulate methane monooxygenase) operons from *Methylocystis* strain SC2. Appl Environ Microbiol 70:3055–3063

Semrau JD, Chistoserdova A, Lebron J, Costello A, Davagnino J, Kenna E, Holmes AJ, Finch R, Murrell JC, Lidstrom al ME (1995) Particulate methane monooxygenase genes in methanotrophs. J Bacteriol 177:3071–3079

Semrau JD, DiSpirito AA, Yoon S (2010) Methanotrophs and copper. FEMS Microbiol Rev 34:496–531

Stoecker K, Bendinger B, Schoning B, Nielsen PH, Nielsen JL, Baranyi C, Toenshoff ER, Daims H, Wagner M (2006) Cohn's Crenothrix is a filamentous methane oxidizer with an unusual methane monooxygenase. Proc Natl Acad Sci USA 103: 2363–2367

Stolyar S, Costello AM, Peeples TL, Lidstrom ME (1999) Role of multiple gene copies in particulate methane monooxygenase activity in the methane-oxidizing bacterium *Methylococcus capsulatus* Bath. Microbiology 145:1235–1244

Stolyar S, Franke M, Lidstrom ME (2001) Expression of individual copies of *Methylococcus capsulatus* bath particulate methane monooxygenase genes. J Bacteriol 183:1810–1812

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Vorobev AV, Baani M, Doronina NV, Brady AL, Liesack W, Dunfield PF, Dedysh SN (2011) *Methyloferula stellata* gen. nov., sp nov., an acidophilic, obligately methanotrophic bacterium that possesses only a soluble methane monooxygenase. Int J Syst Evol Microbiol 61:2456–2463

Whittenbury R, Phillips KC, Wilkinson JF (1970) Enrichment, isolation and some properties of methane-utilizing bacteria. J Gen Microbiol 61:205–218

Yimga MT, Dunfield PF, Ricke P, Heyer H, Liesack W (2003) Wide distribution of a novel pmoA-like gene copy among type II methanotrophs, and its expression in *Methylocystis* strain SC2. Appl Environ Microbiol 69:5593–5602