Expression of Individual Copies of *Methylococcus capsulatus* Bath Particulate Methane Monoxygenase Genes

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The expression of the two gene clusters encoding the particulate methane monoxygenase (pMMO) in *Methylococcus capsulatus* Bath was assessed by analysis of transcripts and by use of chromosomal gene fusions. The results suggest that the two clusters are functionally redundant but that relative expression alters depending on the copper levels available for growth.

Methanotrophic bacteria oxidize their growth substrate methane to methanol via the methane monoxygenase (MMO). Two types of MMO are known, the particulate MMO (pMMO) and the soluble MMO (sMMO) (3). The genes encoding the three subunits of pMMO (*pmoCAB*) are found in multiple copies in methanotrophs (2, 9, 11). In *Methylococcus capsulatus* Bath, a type I γ-proteobacterial methanotroph (3), two complete copies of *pmoCAB* and a third copy of *pmoC* are present (11). Mutant analysis has shown that neither copy of *pmoCAB* is essential but that copy 2 is more important than copy 1 for growth and whole-cell methane oxidation (11). The role of the third copy of *pmoC* is unknown, but it may be essential (11).

In order to assess the relative expression of each set of *pmo* genes under different growth conditions, we have carried out a study of transcripts in *pmoC1* and *pmoC2* mutants and compared these results to expression from promoter-reporter gene fusions in strains containing wild-type *pmoC1* and *pmoC2*.

*Escherichia coli* strains DH5α, DH5α MCR (Bethesda Research Laboratories, Inc.), Invα and Top10 (Invitrogen), and S17-1 (10) were grown in Luria-Bertani medium in the presence of appropriate antibiotics as described previously (8). *M. capsulatus* Bath wild-type and mutant strains (MCK60 and MCK62) (11) were grown as described previously (11).

Transcript analysis. RNA blots were analyzed for insertion mutations of *pmoC1* and *pmoC2* with probes for *pmoC, pmoA*, and *pmoB* (Fig. 1). RNA was isolated from cells using the Perfect RNA total RNA isolation kit (Eppendorf-5 Prime, Inc., Boulder, Colo.). RNA blots were made, and hybridization was carried out at 55°C as described previously (8). The membranes were washed twice with 0.5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 55°C. Hybridization probes were generated from PCR products labeled with a random-primed labeling kit (Boehringer Mannheim). Primers for the reverse transcription-PCR products labeled with a random-primed labeling kit (Boehringer Mannheim, Indianapolis, Ind.). The PCR products were generated from the following primers: *pmoC1* and *pmoC2*, css2F (5′- CCTTGTTGATGGTTGAC-3′) and css2R (5′-GCCTTCGTCCACGGCTTT-3′); *pmoA1* and *pmoA2*, ass1F (5′- CTGGGACTTCTGGTGTCGGACTG-3′) and mb661 (5′- CGGGGTGTAATGCGCTGGTAC-3′).}

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is a highly conserved AT-rich region (CCTCGCGTCAAAATC t/aCTCAg/tATTTTTC). This conserved sequence is a candidate for a regulatory sequence or an upstream promoter element (7). Transcription start sites were determined to be the same for both operons under conditions with and without copper added to the growth medium (data not shown). A promoter for one of the copies of pmoCAB of the type II, α-proteobacterial methanotroph Methylocystis strain M has been mapped, and it also resembled an E. coli σ70 promoter (6). However, it was different from the sequences that we report here and did not contain the AT-rich region.

**Chromosomal reporter gene fusions for pmoC1 and pmoC2.**

Promoter-reporter (xylE) transcriptional fusions were generated in the chromosome for pmoC1 and pmoC2 using a new integrative vector, pMFX1. This vector contains the xylE gene from pHX200 (14) inserted into the KpnI sites of pAYC61 (1), with the Km′ gene from pUC4K replacing the Ap′ gene of pAYC61. This vector can be used to insert promoter-xylE transcriptional fusions into the chromosome at the site of the promoter fragment, as single-crossover insertions generating a fused gene followed by an intact gene with the native promoter. A 1,548-bp fragment containing the pmoC1 promoter region and a 443-bp fragment containing the pmoC2 promoter region were used to generate the chromosomal insertion strains, designated MCX13-2 and MCX215, respectively. Each construct contained a promoter fragment that had the same 3′ end (25 bp of the 5′ region of pmoC), and the remainder was upstream DNA. These constructions were transferred to M. capsulatus Bath by conjugation as described previously (12) and were selected on kanamycin (50 mg/liter). Diagnostic PCR of chromosomal DNA (11) confirmed the expected constructions. These mutants grew at the same rate as the wild type. XylE (catechol dioxygenase) activities (5) were determined in crude extracts of the mutants in 100 mM phosphate.
buffer, which were obtained by passing cells through a French
3
buffer, which were obtained by passing cells through a French
cell pressor and centrifugation for 10
min at approximately 15,000 × g, or in whole cells permeabil-
ized by treatment with 2% (vol/vol) toluene for 30 min (Table
1). The protein concentration was assessed spectrophotometri-
cally (13).

These results show that the copy 2 promoter is expressed at about twice the rate of the copy 1 promoter under normal growth conditions and that promoter activity of both pmoC copies in mid- to late exponential growth (12 to 24 h) is higher in cells grown under normal copper conditions than in cells grown with no added copper. However, for each fusion the amount of reporter activity from cells grown in the absence of added copper was similar to the activity in cells grown with added copper until later in growth, when a 1.5- to 2-fold differ-
ence was observed. These results are qualitatively similar to those of the Northern blot experiments (Fig. 1).

Taken together, the results presented here show that both pmoCAB clusters are transcribed as operons from similar promoters but that transcription of copy 2 dominates under most growth conditions tested. However, it appears that transcrip-
tion of copy 1 increases to a level comparable to that of copy 2 during growth with high copper levels. Since the strains with mutations of both copies show only minor growth defects (11), it seems likely that the two nearly identical copies of pmoCAB in this methanotroph are functionally redundant under the conditions tested, although it is possible that they play different roles in natural habitats.

Nucleotide sequence accession numbers. The GenBank accession number for the fragment upstream of pmoC1 is L40804, and those for the fragment upstream of pmoC2 are U94337 and AF273026.

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REFERENCES


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<th>Strain</th>
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* M. capsulatus strains were grown in either copper-depleted medium (−) or medium containing 20 μM copper (+).

b Experiments were replicated at least twice; each data point is the mean from at least three replicate assays, and the results agreed by ±15%.

TABLE 1. Xyle activity of transcriptional fusions of pmoC in M. capsulatus Bath cells grown under different conditions