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Genetics in Marine Methane-oxidizing Bacteria

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The research objectives of this project are to define the organization and regulation of methanol oxidation genes in a marine methane-oxidizing bacterium (*Methylomonas* sp. A4). Two of the genes have been physically mapped, *mox*F and *mox*I, and expression studies have identified the direction of transcription. The 5' region of *mox*F has been sequenced which has identified a putative promoter sequence. The promoter region for *mox*F will be confirmed and studied in the next year.

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DATE: FEBRUARY 1, 1990

PROGRESS REPORT ON CONTRACT N00014-88-K-0219, R&T CODE 4412039

PRINCIPAL INVESTIGATOR	Mary E. Lidstrom
CONTRACTOR	California Institute of Technology
CONTRACT TITLE	Genetics in Marine Methane-oxidizing Bacteria
START DATE	February 1, 1988

RESEARCH OBJECTIVE: To clone genes involved in one-carbon metabolism from a marine methanotroph, *Methylomonas* sp. A4, and study their regulation at transcriptional and post-transcriptional levels.

PROGRESS (YEAR 2): During the second year of this project, we have concentrated on the physical mapping of C-1 genes from *Methylomonas* A4 and the generation of sequence data necessary for defining promoters.

(1) ***Mapping of Mox genes***

At the time this project was initiated, we had two clones in hand that contained genes involved in methanol oxidation (*mox* genes), one encoding the 60kD subunit of the methanol dehydrogenase (*mox*F) and one encoding a gene involved in cofactor-apoprotein assembly for the methanol dehydrogenase (*mox*A3). We have concentrated on the *mox*F gene, which has been more precisely mapped, and the direction of transcription has been deduced by expression in *E. coli* using a T7 polymerase/promoter expression system. The expression studies also revealed that another Mox gene, (*mox*I) encoding the 10kD subunit of the methanol dehydrogenase, was present on this clone, transcribed in the same direction as *mox*F and downstream approximately 4kb. The identity of these proteins was confirmed by Western blotting. We are currently generating a new series of T7 subclones to more precisely locate the coding region for the small subunit.

(2) ***Sequencing***

In order to analyze the *mox*F promoter, we must have sequence data for both the 5' portion of the ORF and the upstream region. This sequencing was initiated with a primer synthesized from a tetrapeptide (at residues 13-16 of the mature protein) that is conserved in all five known Mox F sequences (see Fig. 1). We are currently investigating the use of this oligomer as a possible gene probe or PCR primer for detection of *mox*F sequences. The translational start site for MoxF has been identified by comparison of the DNA sequence with the amino acid sequence of the mature protein, which we had determined previously. MoxF is known to be a periplasmic protein, and translation of the DNA sequence has identified a leader sequence of size similar to those for other MoxF proteins (30 aa) with a cleavage site immediately after A-E-A. This signal sequence has the standard hydrophilic-hydrophobic structure, with a proline at residue 27. The N-terminal aa sequence (120 aa) is highly similar (81% similarity) to the sequence we have obtained from another *Methylomonas* species, but shows more divergence from MoxF sequences of methanol utilizers (74% similarlity). We have also obtained some C-terminal sequence data and have identified a stretch of 150 aa that shows 81% similarity with other known sequences. Clearly this protein is highly conserved in diverse methylotrophs, and it should be possible to design gene probes and

PCR primers for detection of *mox*F in environmental samples. Comparison to the sequence for another alcohol dehydrogenase quinoprotein that does not use methanol, from *Acetobacter aceti* shows low similarity (15-20%) and the conserved tetrapeptide used to design the primer noted above is not present in this sequence. Therefore, this oligomer should be specific for *mox*F and should not identify other quinoprotein genes. This hypothesis will be confirmed by screening studies.

The region upstream of the translational start site contains a good ribosome binding site sequence, and approximately 85 bp upstream a sequence is present that resembles other putative MoxF promoters (see Fig. 2). From information obtained in our laboratory as well as others, we have proposed a "consensus" Mox promoter, and this sequence fits the consensus quite well (see Fig. 2). We are currently carrying out experiments to determine whether this is indeed the *mox*F promoter in *Methylomonas* A4.

WORK PLAN (YEAR 3): First, we intend to map the transcriptional start site for *mox*F. Although no protocol exists for isolating mRNA from a methanotroph, we have a new miniprep procedure that has been successful in another *Methylomonas* strain in the laboratory, and so we are optimistic that we will obtain mRNA preparations of sufficient quality for primer extension analysis. We also hope to obtain Northern blots, to determine whether this region is transcribed as an operon, as in *Methylobacterium extorquens* AM1, or singly, as in *Methylobacterium organophilum* XX. Once the promoter region is indicated from the transcriptional start site mapping, we will then generate subclones of the 5' regions in pDN19*lac*, a broad-host range promoter probe vehicle, and identify promoter regions by β-galactosidase assays.

PUBLICATIONS FROM THIS PROJECT

Lidstrom, M.E. 1988. Isolation and characterization of marine methanotrophs. Ant. v. Leeuw. J. Microbiol. 54:189-199.

A.A. DiSpirito, J.D. Lipscomb and M.E. Lidstrom. Soluble cytochromes from the marine methanotroph, *Methylomonas* sp. A4. submitted to J. Bacteriol.

A.A. DiSpirito. Soluble cytochrome *c*'s from *Methylomonas* A4. Meth. Enz. vol. 188 (Hydrocarbons and Methylotrophy), in press.

A.A. DiSpirito, D. Waechter-Brulla and M.E. Lidstrom. Cloning of methanol oxidation (Mox) genes from the marine methanotroph, *Methylomonas* A4. to be submitted to J. Bacteriol.

INVENTIONS: None

PERSONNEL SUPPORT: This grant has supported the salary of Daryle Waechter-Brulla, a post-doctoral fellow. In addition, Bill Brulla, a senior technician and Alan DiSpirito, a senior research fellow have received partial salary support from this grant and have contributed to the results presented.

FIGURE 1

NH2-TERMINAL AMINO ACID SEQUENCES OF moxF

```
AM1  DNA   A   NDKLVELSKSDDNWVMPGKDYDSNNPSDLKQ
XX   DNA   A   NDKLVELSKSDDNWVMPGKDYDSNNYSDLKQ
PD   DNA   A   NDQLVELAKDPANWVMTGRDYNAQNTSEMTD
BG8. DNA   A   NKELDRLSKEDINWVMQTKDYGATHFSKMTQ
A4   DNA   A   NKELDQMSRQNTNWVMQTKDYSSTHYSELYD
A4    AA       NKELDQMSRQNTNWVMQT
```

FIGURE 2

COMPARISON OF UPSTREAM SEQUENCES FOR *MOX* F AND *DCM* A GENES

	-35		-10	+1
E. COLI	TTGACA	XXXXXXXXXXXXXXXXX	TATAAT	XXXXX*G*
AM1	CT **AAAGACA**	TCGCGTCCAATCAAAGCC	**TAGAAA**	ATATA*G*---170bp--- first Met
XX	GT **AAAGACA**	TCTCCTTCAATCAACGCC	**TAGAAA**	CGAT*A* ---171bp--- first Met
DM4	AG CTT**GACA**	GAGATGCATAGCCTTGTA	**TAGAA**C	TAGC*C*---72bp--- first Met
BG8	AA **AAAG**GA**A**	CTTTTCCCGACTCACA	CG**GAAA**	AGCCAT*A* ---212bp--- first Met
A4	AA **AAA**TC**CA**	AGGCATACGTTTTGATC	A**AGAAA**	AGCTGAT ---85bp--- first Met
Pd		GATCGGAC	GG**GAAA**	AACCC---157bp--- first Met

AM1 = *Methylobacterium extorquens* AM1 (serine pathway methanol-utilizer)
XX = *Methylobacterium organophilum* XX (serine pathway methanol-utilizer)
DM4 = *Methylobacterium* sp. DM4 (utilizes dichloromethane)
Pd = *Paracoccus denitrificans* (autotrophic methanol-utilizer)
BG8 = *Methylomonas albus* BG8 (RuMP pathway methane-utilizer)
A4 = *Methylomonas* sp. A4 (marine RuMP pathway methane-utilizer)

PUTATIVE CONSENSUS PROMOTER SEQUENCE FOR METHYLOTROPHS

-35		-10	
AAAG**A**C**A**	--16-18 bases--	T**AGAAA**	--5-7 bases--start site