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The methanol oxidation genes *mxaFJGIR(S)ACKLD* in *Methylobacterium extorquens*

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Abstract

MxaJ is a protein of unknown function encoded by *mxaJ* in the *mxaFJGI* operon. We have constructed a *mxaJ* mutant of *M. extorquens* with a deletion which does not affect transcription of downstream genes. It contained cytochrome c_L (MxaG), but neither subunit of methanol dehydrogenase (MxaF and MxaI). MxaJ is probably involved in processing this enzyme. We have sequenced the region between *mxaFJGI* and five other methanol oxidation genes, *mxaACKLD*; it includes one open reading frame (*mxaR*) and a possible second open reading frame (*mxaS*), demonstrating the presence in *M. extorquens* of the following gene cluster: *mxaFJGIR(S)ACKLD*.

Keywords: Methanol oxidation; *Methylobacterium extorquens*; *mxaFJGIR(S)ACKLD* operon

1. Introduction

In Gram-negative methylotrophic bacteria, methanol is oxidised by methanol dehydrogenase (MDH), a quinoprotein with an $\alpha_2\beta_2$ tetrameric structure, containing pyrrolo-quinoline quinone (PQQ) and a calcium ion at its active site. Its electron acceptor is a specific *c*-type cytochrome, usually designated cytochrome c_L [1,2]. There are at least 30 methanol oxidation genes, arranged in 5 clusters; these include the genes involved in PQQ biosynthesis [2]. The genes encoding the α and β subunits of MDH (*mxaF* and *I*) and cytochrome c_L (*mxaG*) are situated in an operon together with *mxaJ* [3–5]. This encodes a 30-kDa periplasmic protein suggested to be either a third (non-essential) subunit of MDH [6] or a molecular chaperone [5].

The genes *mxaFJGI* are transcribed from a promoter upstream of *mxaF*; this is the only promoter so far definitively identified in a methylotroph [4,7]. About 2 kb downstream from *mxaI* in *Methylobacterium extorquens* is another cluster of genes (*mxaACKLD*) some, if not all, of which are involved in the insertion of calcium into the active site of MDH [8,9]. The *mxaACKLD* cluster has not been described in *Paracoccus denitrificans*, but immediately downstream of *mxaI* in this organism there is another gene (*mxaR*) and the 3′ end of a putative open reading frame subsequently designated *mxaS* [5,9]. Studies with *mxaJ* mutants have shed little light on the role of this gene [5,10] because interpretation of the phenotype is difficult as the mutations may affect expression of neighbouring genes.

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In this paper we describe the construction of a mutant of *M. extorquens* with a deletion in *mxaJ* which does not have any confusing effects on the transcription of downstream genes. We have also completed the sequencing of the region between *mxaI* and *mxaA*; this indicates that there are 10 or 11 genes in this cluster, in the order *mxaFJGIR-(S)ACKLD*.

2. Materials and methods

The bacterial strains and plasmids used in this study are shown in Table 1. *Escherichia coli* strains were grown at 37°C in LB medium [11]. *M. extorquens* AM1 was grown at 30°C on minimal salts medium containing 0.5% methanol plus methylamine (0.4%) as described in [12]. Supplements were added to the growth medium when appropriate to give the following final concentrations: for solid medium kanamycin, 50 μg ml^{-1}; ampicillin, 50 μg ml^{-1}; tetracycline, 20 μg ml^{-1}: for liquid medium antibiotic concentrations were halved. When X-gal was used, 20 μl of a 50 mg ml^{-1} stock solution in dimethylformamide (Promega) was spread on each agar plate just before plating.

M. extorquens chromosomal DNA was purified as described previously [13]. The Wizard™ miniprep DNA purification system (Promega) was used for small-scale plasmid isolation from *E. coli*. Large-scale plasmid preparation by the alkaline lysis method, DNA manipulations, transformation of *E. coli*, and agarose gel electrophoresis were done as described previously [11]; restriction enzymes were obtained from Promega or New England Biolabs. Southern blotting was done as described in the Hybaid Limited Hybridisation Guide (1992). Probes were labelled with [α-^{32}P]dCTP and random hexamers using the Prime-a-Gene kit (Promega). Bacterial matings were done as described in [13] except that donors and recipients were grown as lawns on solid medium. The bacteria were scraped off, mixed in a 1:5 donor:recipient ratio and plated onto minimal salts medium containing methanol and methylamine.

Single and double stranded DNA was sequenced by the dideoxy chain termination method using the Sequenase® version 2.0 kit (USB) with the 7-deaza-dGTP nucleotide mixtures, using specific custom-made primers. Oligonucleotide primers were synthe-

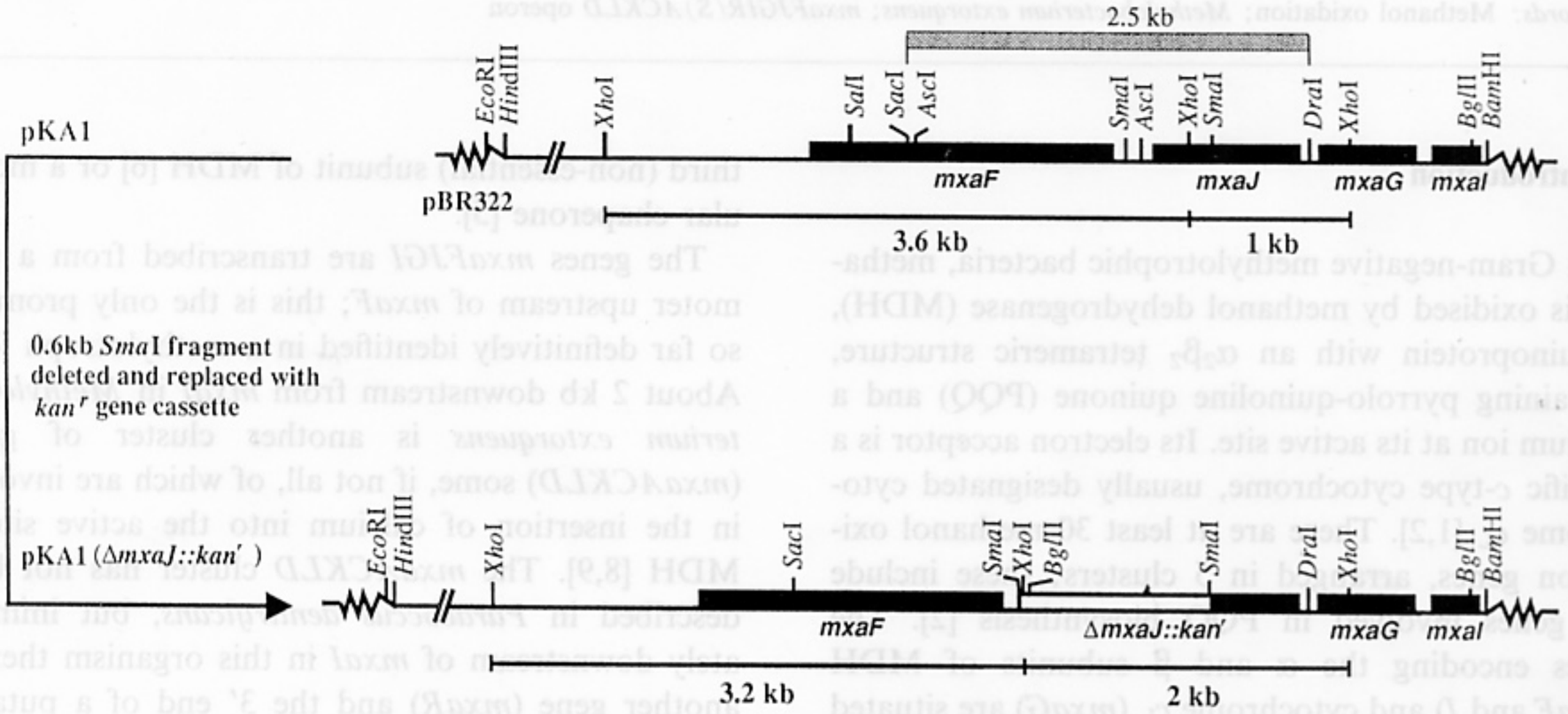

Fig. 1. Restriction map of the *mxaFJGI* operon from *M. extorquens* and strategy for construction of the *mxaJ* deletion mutant. The map is not strictly to scale. Transcription of the *mxa* genes is from left to right. The 0.6 kb deletion within *mxaJ* is shown. The white box indicates the kanamycin resistance reporter gene replacing the deleted DNA. The probe used for Southern hybridization is shown as a grey box. Bars below each restriction map show the wild-type and mutant *Xho*I fragments hybridizing to the probe. The *Eco*RI site is within the pBR322 vector sequence. The *Xho*I fragments from the wild-type (1 kb+3.6 kb) and the KAJ7 (Δ*mxaJ::kan*^{r}) mutant sequences (2 kb+3.2 kb) hybridised with the T_4 probe in Southern blotting experiments as expected.

Table 1
Bacterial strains and plasmids

Strain or plasmid	Relevant properties[a]	Source/Reference
M. extorquens		
Wild-type	Rif^r	[18]
Cou-3	*mxaJ*::Tn*5* (*kan*^{r})	[10]
KAJ7 (Δ*mxaJ::kan*^{r})	0.6-kb deletion in *mxaJ* replaced by *kan*^{r} gene	This study
E. coli		
HB101	F^-, *leuB6, proA2, recA13, thi1, ara14, lacY1, galK2, xyl5, mtl1, supE44, hsd20* (r_B^-, m_B^-), Str^r	[22]
S17-1	*thi, pro, hsdR*$^-$, *hsdM*$^-$, *recA*, RP4-2 integrated (Tc::Mu)(Km::Tn*7*)	[23]
TB1	F^-, *ara*, Δ*lac-proAB, rpsL*, ϕ80, *lacZ*Δ*M15, hsdR* (r_K^-, m_K^+)	BRL
Plasmids		
pBR322	*amp*^{r}, *tet*^{r}	Promega
pUC4-KIXX	*amp*^{r} *kan*^{r} gene cassette vector	Pharmacia
pVK100	*tet*^{r}, *kan*^{r} IncP1 cosmid	[24]
pVK100 (*mxaFJGI*)	pVK100 with an 8.6-kb *Hin*dIII-*Hin*dIII fragment containing *mxaFJGI* inserted in the *kan*^{r} gene	[18]
pKA0	pBR322 lacking the *Sal*I site. *amp*^{r}	This study
pKA1	6.8-kb *mxaFJGI Hin*dIII-*Bam*HI fragment inserted in pKAO. *amp*^{r}	This study
pKA1 (Δ*mxaJ*)	pKA1 with 0.6kb deletion in *mxaJ*. *amp*^{r}	This study
pKA1 (Δ*mxaJ::kan*^{r})	*kan*^{r} gene cassette inserted in *mxaJ*.	This study
pRS415	*amp*^{r}, *lacZ*$^+$ *Y*$^+$ *A*$^+$, pBR322 backbone	[25]
pKA415J	pRS415 containing Δ*mxaJ::kan*^{r} *Hin*dIII-*Bam*HI fragment. *amp*^{r}, *kan*^{r}, *lac*$^+$	This study
M13mp18HB	M13mp18 with 1.5 kb *Hin*dIII-*Bam*HI fragment of region downstream of *mxaI*	This study
M13mp19HB	M13mp19 with 1.5 kb *Hin*dIII-*Bam*HI fragment of region downstream of *mxaI*	This study

[a] *amp*^{r}, ampicillin-resistant; *kan*^{r}, kanamycin-resistant; *rif*^{r}, rifampicin-resistant; *str*^{r}, streptomycin-resistant; *tet*^{r}, tetracycline-resistant; IncP1, incompatibility group P1.

sised, with the trityl group attached, using a model 391 PCR-MATE™ DNA synthesiser on a 40 nmole scale. The synthetic DNA was purified using oligonucleotide purification cartridges (Applied Biosystems). Some compressions were resolved by adding 40% formamide to the sequencing gels. DNA fragments were cloned into M13mp18 in one direction and into M13mp19 in the opposite direction, to facilitate sequencing of both strands. The sequence was also confirmed in both directions by automated DNA sequencing on a Li-Cor model 4000 sequencer. DNA and protein sequences were analysed using PC/Gene (IntelliGenetics) and DNASTAR (DNASTAR Inc., Wisconsin, USA) and compared with sequences in the GenBank, Swiss-Prot and Prosite databases using the FASTA and BLAST programs [14,15].

Respiration [16], cell extract preparation and MDH assays [12], SDS-PAGE of cell extracts and haem staining [17] were done as previously described using at least two independent cultures. For SDS-PAGE (for protein analysis), cell extracts (150 μg protein) were mixed with 0.2 volumes of loading buffer, boiled for 5 min and spun briefly before loading. For SDS-PAGE when subsequently haem staining, samples were mixed with loading buffer in the absence of β-mercaptoethanol and were loaded without boiling. For Western blotting, proteins were transferred from SDS-PAGE gels to nitrocellulose using the Bio-Rad mini Trans-Blot system. Rabbit anti-holoMDH, anti-MDH β-subunit and anti-cytochrome c_L were used as primary antibodies. Horseradish peroxidase-conjugated donkey anti-rabbit IgG (Bio-Rad) was used as secondary antibody.

3. Results and discussion

3.1. Construction and characterisation of a marked, non-polar deletion mutant (mutant KAJ7) in mxaJ in Methylobacterium extorquens

The strategy used for the construction of the dele-

```
GGATCCGTCCTGACCTGCCGGATCGGGCAGGCCCGATCCGGAGCGGGAGGGGCGGACACC - 60
GCGTGAGGGCGCTCCCAACGACAAGAAAAAGGCCAACCGCCGAATGAACACCTTGCGCCC - 120
                                MxaR:- M  N  T  L  R  P

CGGCGACGCCATGCTCACCGACTGGCGGGACGCGGCCGCGCGTTTCGAGCGCGAGATCGC - 180
  G  D  A  M  L  T  D  W  R  D  A  A  A  R  F  E  R  E  I  A
     *              *  *                       *

CAAGGCCGTCGTCGGTCAGGACCGGGCGATCCGCCTGCTGACGATCGCGATTTTCGCCCG - 240
  K  A  V  V  G  Q  D  R  A  I  R  L  L  T  I  A  I  F  A  R
        *  *  *     *     *  *  *  *     *              *

CGGCCACGTCATGCTCGAAGGCGATGTCGGCGTCGGCAAGACCACGCTGCTGCGCGCGGT - 300
  G  H  V  M  L  E  G  D  V  G  V  G  K  T  T  L  L  R  A  V
  *  *  *     *     *  *  *  *  *  *  *  *  *  *  *  *  *  *

CGCCCGCGGCCTCGGCGGCGCCTACGAGCGCGTCGAGGGCACCGTCGACATGATGCCCAC - 360
  A  R  G  L  G  G  A  Y  E  R  V  E  G  T  V  D  M  M  P  T
  *  *     *  *  *     *     *  *  *  *  *  *  *        *  *

TGACCTGATCTATCACACCTATCTCGGCGAGGATGGCCGCCCGCGGGTCGAGCCCGGCCC - 420
  D  L  I  Y  H  T  Y  L  G  E  D  G  R  P  R  V  E  P  G  P
  *  *  *  *     *           *  *  *  *  *  *     *  *  *  *

GGTGCTGCGGCGCGCCGAAGATCTGTCGGTGTTCTTCTTCAACGAGATCAACCGCGCCCG - 480
  V  L  R  R  A  E  D  L  S  V  F  F  F  N  E  I  N  R  A  R
  *  *           *  *        *  *  *  *  *  *  *  *  *  *  *

GCCGCAGGTCCACGCGCTGCTCCTGCGCATCATGGCCGAGCGCAGCGTCAGCGCCTTCAA - 540
  P  Q  V  H  A  L  L  L  R  I  M  A  E  R  S  V  S  A  F  N
  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

CCGCGAGTACCGCTTCCCCAACCTCCAGGTCTTCGCCGACCGCAACCGCGTCGAGCGCGA - 600
  R  E  Y  R  F  P  N  L  Q  V  F  A  D  R  N  R  V  E  R  E
  *  *  *  *  *  *     *  *  *  *  *  *  *  *        *  *

GGAGACCTTCGAGCTGCCGGCCGCCGCCCGCGACCGCTTCCTCATGGAGATCGGCATGGA - 660
  E  T  F  E  L  P  A  A  A  R  D  R  F  L  M  E  I  G  M  E
  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

GGCGCCGCGCGACGCCGCCCGCCGCGACCTCGTGTTCGATCCCCGCTTCCACGACACCGA - 720
  A  P  R  D  A  A  R  R  D  L  V  F  D  P  R  F  H  D  T  D
  *  *              *     *     *  *  *  *  *  *

CCGGCTCACCGAGGAGGTCGAGGCCGGCGTGCTCGACTTCGAGCGCATCGGCACCATCGC - 780
  R  L  T  E  E  V  E  A  G  V  L  D  F  E  R  I  G  T  I  A
     *     *  *     *     *                    *

CAGCGCGATCCAGCACGCGATCTCGGCCGAGCCGGCGATCGAGGCCTACGTCGTCGGCCT - 840
  S  A  I  Q  H  A  I  S  A  E  P  A  I  E  A  Y  V  V  G  L
        *           *     *  *           *  *           *

GTGGGAGGCGCTGGTGCGCCCGGCCCCGCCGCATCCGCTTGCCCGGCATCGCATGGACCG - 900
  W  E  A  L  V  R  P  A  P  P  H  P  L  A  R  H  R  M  D  R
     *  *  *           *  *              *              *

CCTCGTCCAGGGCGGCGCCTCGCCCCGCGGCGTCGCCTTCCTCGTGCGCGCCGCCCGCGT - 960
  L  V  Q  G  G  A  S  P  R  G  V  A  F  L  V  R  A  A  R  V
  *     *  *     *  *  *  *              *  *  *  *  *  *

CCGCGCTTGGCTGGAAGGCCGGGACTGGCTGGTGCCGGAGGATATCCGCGCCGTCTTCCC - 1020
  R  A  W  L  E  G  R  D  W  L  V  P  E  D  I  R  A  V  F  P
  *  *  *  *  *  *                 *  *  *  *  *
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CCGCGTCTTCCTCGAACCCGTCTACGAGATGCGCCGCGCCGAGAT - 1080
  R  V  F  L  E  P  V  Y  E  M  R  R  A  E  I
  *     *  *     *     *  *     *  *

CCGCGCGGTGTTCGAGACGGTGCCCGCCCCGTGAGCGGTCCGGAG - 1140
  R  A  V  F  E  T  V  P  A  P  -
     *     *     *     *     *

GGGGCCGACATCATCTACCTCCCGCGCTGGCGGCCGGGCGGGCAC - 1200
CGCGGGCCGGATGCCGGCGGTCTCGGCACGTTTCGCGATCAGGTC - 1260
CCGGATGGCGGGATCGACGTGCGCGCGTGCTGCGCGACCCGTTCG - 1320
GCGCTTCGAGGCGGCAGCCGGTCGAGACCTACGCGCTGGTCGATC - 1380
CTTCCGCGGCCGGGCCGACCGGCGCGAACTGGCGGCCGGGTTCTG - 1440
A  S  A  A  G  P  T  G  A  N  W  R  P  G  S

CTCGCGACGCGGATCGGCGACGGCTTCGGCCTGATCGCCTGCGAC - 1500
A  R  D  A  D  R  R  R  L  R  P  D  R  L  R

GACCTCACCCTCCCCGCGACCCGCCACCGGGCCGCCGCCCAGGCC - 1560
R  P  H  P  P  R  D  P  P  P  G  R  R  P  G

GGCGAGGCGGCCTGCGACGGACGCGGCGCCGAGGGGCTGCTGGAG - 1620
R  R  G  G  L  R  R  T  R  R  R  G  A  A  G

CCGGGCGCCCGAAGCTGATCTTCCTCGTCTCGGATTTCCGCTGGC - 1680
A  G  R  P  K  L  I  F  L  V  S  D  F  R  W
              *     *     *  *  *

AGGCGGTGTTCTCGGCGCTGGCGCTGCACGATGTGACCCCGGTGC - 1740
E  A  V  F  S  A  L  A  L  H  D  V  T  P  V
   *        *  *              *        *

CCGAGGCCGAAGACCTGCCGAACTGGGGCCTCGTGGAACTCGACG - 1800
A  E  A  E  D  L  P  N  W  G  L  V  E  L  D
               *        *  *     *  *

GGCGCCGCCTCGTCTTCCTGCGCCCATCCTTGCGCCGCCGCTGGA - 1860
G  R  R  L  V  F  L  R  P  S  L  R  R  R  W
   *  *     *        *              *  *

AGCGCGTCGAAGCTTTGCGCCGGATCTGCGCGCCCTTCGCCCGTC - 1920
E  R  V  E  A  L  R  R  I  C  A  P  F  A  R
   *     *  *  *  *  *     *

CCGACCGCTTCGACGCCGAGGCGCTGAGCCGCCACCTGATGACGA - 1980
A  D  R  F  D  A  E  A  L  S  R  H  L  M  T
```

Fig. 2. The nucleotide sequence of the region between *mxaI* and *mxaA* in *M. extorquens*. Nucleotides 1–344 and the sequence downstream of 1886 have been described previously [9,20]. A possible Shine-Dalgarno site upstream of *mxaR* is underlined. The protein sequence labelled in parentheses (MxaS) represents the predicted translation product of the only open reading frame between *mxaR* and *mxaA*. The protein sequence that is in italics and underlined represents the position of the start of the putative MxaS of *P. denitrificans*. The Mg-ATP-binding Walker motifs A and B are underlined. The consensus sequence of motif A is pXXXXGXXGXGKT where p is a positively charged amino acid. The consensus sequence of motif B is RXXGXXXLfffD, where f is a hydrophobic residue. Asterisks indicate amino acids that are identical in the predicted proteins in *P. denitrificans*.

tion mutant in *mxaJ* is outlined in Fig. 1. The *Sal*I site of pBR322 was removed by repair of cohesive ends with T4 DNA polymerase giving pKA0. The 6.8 kb *Hin*dIII-*Bam*HI fragment of a pVK100 derivative, containing the *mxaFJGI* operon [18], was cloned into pKA0, giving pKA1. To construct the *mxaJ* deletion, the 0.6 kb *Sma*I fragment of *mxaJ* was replaced by the kanamycin resistance gene from pUC4-KIXX [19]. This kanamycin resistance gene cassette was chosen because it did not contain tran-

scription terminators which would otherwise prevent transcription of *mxa* genes downstream of *mxaJ*. The orientation of the kanamycin resistance gene was determined by restriction digests with *Bgl*II (Fig. 1). A clone carrying a kanamycin resistance gene which would be transcribed in the same direction as *mxaFJGI* was selected to construct the mutant.

Using the *Eco*RI site in the pBR322 sequence immediately upstream of the *Hin*dIII site which was used to construct pKA1, the *Eco*RI-*Bam*HI fragment from pKA1 (Δ*mxaJ::kan*^{r}) was cloned into the suicide vector pRS415, a pBR322 derivative, which cannot replicate in *M. extorquens*. The resulting construct, pKA415J, was first transformed into *E. coli* TB1, to confirm that clones still expressed the plasmid-borne *lacZ* gene, and then mobilised into wild-type *M. extorquens* AM1 from *E. coli* S17-1. Transconjugants were plated onto methanol medium containing kanamycin and X-gal to select for colonies which had arisen as a result of integration of the plasmid into the chromosome by a single crossover. These were then streaked onto nutrient agar containing kanamycin and X-gal to identify Lac$^-$ clones in which the vector and wild-type *mxaJ* gene were subsequently lost by a double crossover. Twelve pink, kanamycin-resistant colonies which were unable to grow on methanol were isolated and Southern blotting of the chromosomal DNA from one of these clones confirmed that 0.6 kb had been deleted from the *mxaJ* gene and replaced by the kanamycin resistance gene.

Mutant KAJ7 had the growth properties expected of a *mxaJ* mutant; that is, it was unable to grow on methanol but could utilise methylamine as sole source of carbon and energy. Whole cells oxidised formaldehyde, methylamine and pyruvate, but not methanol. Extracts contained no methanol dehydrogenase as determined in the dye-linked assay, and neither the α nor the β subunit of this enzyme was detected by Western blotting. However, both haem staining and Western blotting showed that cytochrome c_L was present in the mutant at wild-type levels, demonstrating that genes downstream of the deletion could be expressed. The absence of the β subunit is consistent with previous observations which suggested that its very small size (8.5 kDa) rendered it unstable in the absence of the large α subunit [3]. The phenotype of mutant KAJ7 is quite different from that of the *P. denitrificans mxaJ* deletion mutant which synthesised the α subunit of MDH and also the β subunit and cytochrome c_L (albeit at decreased levels). By contrast, both the *mxaJ* transposon mutant of *M. extorquens* [10] and mutant KAJ7 failed to produce the α subunit of MDH. It is unlikely that MxaJ has a substantially different role in these two methylotrophs and our data are not inconsistent with the suggestion that this protein is involved in the processing of MDH [5]. However, it does appear that the α subunit of MDH in *M. extorquens* is unstable in the absence of MxaJ. In *P. denitrificans* the newly formed MxaF and MxaI polypeptides may be intrinsically more stable, or another protein may be able to take over part of the function of MxaJ.

3.2. The DNA sequence between mxaI and mxaA in Methylobacterium extorquens

The sequence of the 2 kb *Bam*HI-*Hin*dIII fragment which lies between *mxaI* and *mxaA* in *M. extorquens* has been deposited in the EMBL database (accession number Y07864); it overlaps a small amount of previously published sequence downstream from *mxaI* [20] and upstream from *mxaA* [9]. This completes a 11 801-bp sequence and demonstrates that their are two putative reading frames between the *mxaFJGI* cluster and the *mxaACKLD* cluster in *M. extorquens*. The predicted amino acid sequence of the first ORF has 60% identity with MxaR of *P. denitrificans* (Fig. 2). There is no obvious signal peptide or hydrophobic membrane-spanning regions, and the sequence indicates that MxaR (38.6 kDa) is a typical cytoplasmic protein. As no other proteins in the databases have significant homology with MxaR from these two organisms, the only information regarding its function which can be deduced from its sequence is the presence of two motifs relevant to MgATP binding. The first of these is the glycine-rich P-loop (Walker A sequence) [21] involved in binding ATP or GTP; the second motif is also found in some ATP-binding proteins but not in GTP-binding proteins [21]. It can reasonably be concluded, therefore, that the MxaR protein has a function involving binding ATP. The properties of a *mxaR* insertion mutant of *P. denitri-*

ficans suggest that it may be involved in the processing of MDH in the cytoplasm, or in the regulation of other *mox* genes [5].

Our sequence indicates that in *M. extorquens* there is a single open reading frame after that encoding MxaR, which would code for a protein of 22.3 kDa. The C-terminal part shows 37% identity with the putative MxaS of *P. denitrificans* but there was no similarity to any other protein in the databases (Fig. 2). In *M. extorquens,* in the 228-bp region at the start of the putative ORF, the codon usage is completely atypical and the extra predicted 76 N-terminal amino acids would show a remarkable and highly unlikely amino acid composition, containing high proportions of arginine and proline but no glutamate and very little lysine (Fig. 2). Clearly this is not a coding region. There is, however, no initiation codon (ATG or GTG) in the sequence corresponding to the start of *mxaS* in *P. denitrificans*. This is unlikely to be due to a mistake in sequencing because the whole of the 2 kb were sequenced on both strands by both manual and automated sequencing, in duplicate. In the absence of any other evidence, the nature of the sequence identified as *mxaS* in *M. extorquens* should be interpreted with caution and we have consequently placed *mxaS* in parentheses in the sequence of genes proposed for this coding region. The sizes of the genes and intergenic regions (in parentheses) in the *mxaFJGIR(S)ACKLD* cluster are shown below (values here are for *M. extorquens*):

```
---F----------J----------G----------I----------R----------(S)------A--------C--------K--------L--------D-----
1881 (261)  903 (142)  594 (105) 291 (151) 1023 (264) 591 (8) 921 (7) 1065 (3) 624 (0) 1008 (3) 528
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The genes form two well-defined groups in both *M. extorquens* and *P. denitrificans*. The intergenic regions in the first half of the cluster (between *mxaF* and *mxaR*) are relatively large (105–261 bp), whereas those in the downstream half (between the putative *mxaS* and *mxaD*) are very small (0–8 bp). This draws attention to the possibility that this whole cluster might be organised into two operons. However, there is no obvious promoter between *mxaR* and *mxaA* and further work is required to ascertain whether or not the *mxaFJGIR(S)ACKLD* cluster constitutes more than one transcriptional unit.

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Erratum

Erratum to "The methanol oxidation genes *mxaFJGIR(S)ACKLD* in *Methylobacterium extorquens*"

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Fig. 2 of this paper (pp. 35 and 36) has been printed incorrectly. The figure should have appeared as printed on the following two pages.

The Publisher apologizes for any inconvenience this may have caused.

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GGATCCGTCCTGACCTGCCGGATCGGGCAGGCCCGATCCGGAGCGGGAGGGGCGGACACC - 60
GCGTGAGGGCGCTCCCAACGACAAGAAAAAGGCCAACCGCCGAATGAACACCTTGCGCCC - 120
                                        MxaR:- M  N  T  L  R  P

CGGCGACGCCATGCTCACCGACTGGCGGGACGCGGCCGCGCGTTTCGAGCGCGAGATCGC - 180
  G  D  A  M  L  T  D  W  R  D  A  A  A  R  F  E  R  E  I  A
     *              *  *                       *

CAAGGCCGTCGTCGGTCAGGACCGGGCGATCCGCCTGCTGACGATCGCGATTTTCGCCCG - 240
  K  A  V  V  G  Q  D  R  A  I  R  L  L  T  I  A  I  F  A  R
        *     *  *     *     *  *  *  *     *              *

CGGCCACGTCATGCTCGAAGGCGATGTCGGCGTCGGCAAGACCACGCTGCTGCGCGCGGT - 300
  G  H  V  M  L  E  G  D  V  G  V  G  K  T  T  L  L  R  A  V
  *  *  *     *     *  *  *  *     *  *  *  *  *  *  *  *  *

CGCCCGCGGCCTCGGCGGCGCCTACGAGCGCGTCGAGGGCACCGTCGACATGATGCCCAC - 360
  A  R  G  L  G  G  A  Y  E  R  V  E  G  T  V  D  M  M  P  T
  *  *     *  *  *     *     *  *  *  *  *  *  *        *  *

TGACCTGATCTATCACACCTATCTCGGCGAGGATGGCCGCCCGCGGGTCGAGCCCGGCCC - 420
  D  L  I  Y  H  T  Y  L  G  E  D  G  R  P  R  V  E  P  G  P
  *  *  *  *     *           *  *  *  *  *  *     *  *  *  *

GGTGCTGCGGCGCGCCGAAGATCTGTCGGTGTTCTTCTTCAACGAGATCAACCGCGCCCG - 480
  V  L  R  R  A  E  D  L  S  V  F  F  F  N  E  I  N  R  A  R
  *  *           *  *           *  *  *  *  *  *  *  *  *  *

GCCGCAGGTCCACGCGCTGCTCCTGCGCATCATGGCCGAGCGCAGCGTCAGCGCCTTCAA - 540
  P  Q  V  H  A  L  L  L  R  I  M  A  E  R  S  V  S  A  F  N
  *  *  *  *  *  *  *  *  *     *  *  *  *  *     *  *  *

CCGCGAGTACCGCTTCCCCAACCTCCAGGTCTTCGCCGACCGCAACCGCGTCGAGCGCGA - 600
  R  E  Y  R  F  P  N  L  Q  V  F  A  D  R  N  R  V  E  R  E
  *  *  *  *  *  *     *  *  *  *  *  *  *  *        *  *

GGAGACCTTCGAGCTGCCGGCCGCCGCCCGCGACCGCTTCCTCATGGAGATCGGCATGGA - 660
  E  T  F  E  L  P  A  A  A  R  D  R  F  L  M  E  I  G  M  E
  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

GGCGCCGCGCGACGCCGCCCGCCGCGACCTCGTGTTCGATCCCCGCTTCCACGACACCGA - 720
  A  P  R  D  A  A  R  R  D  L  V  F  D  P  R  F  H  D  T  D
  *  *                 *     *     *     *  *  *  *  *  *

CCGGCTCACCGAGGAGGTCGAGGCCGGCGTGCTCGACTTCGAGCGCATCGGCACCATCGC - 780
  R  L  T  E  E  V  E  A  G  V  L  D  F  E  R  I  G  T  I  A
     *        *  *        *     *                       *

CAGCGCGATCCAGCACGCGATCTCGGCCGAGCCGGCGATCGAGGCCTACGTCGTCGGCCT - 840
  S  A  I  Q  H  A  I  S  A  E  P  A  I  E  A  Y  V  V  G  L
           *                 *     *  *           *  *        *

GTGGGAGGCGCTGGTGCGCCCGGCCCCGCCGCATCCGCTTGCCCGGCATCGCATGGACCG - 900
  W  E  A  L  V  R  P  A  P  P  H  P  L  A  R  H  R  M  D  R
     *  *  *        *  *                 *                 *

CCTCGTCCAGGGCGGCGCCTCGCCCCGCGGCGTCGCCTTCCTCGTGCGCGCCGCCCGCGT - 960
  L  V  Q  G  G  A  S  P  R  G  V  A  F  L  V  R  A  A  R  V
  *        *  *     *  *  *  *           *  *  *  *  *  *

CCGCGCTTGGCTGGAAGGCCGGGACTGGCTGGTGCCGGAGGATATCCGCGCCGTCTTCCC - 1020
  R  A  W  L  E  G  R  D  W  L  V  P  E  D  I  R  A  V  F  P
  *  *  *  *  *  *           *     *     *  *  *
```

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CGAGGTGATGGCCCACCGCGTCTTCCTCGAACCCGTCTACGAGATGCGCCGCGCCGAGAT - 1080
  E  V  M  A  H  R  V  F  L  E  P  V  Y  E  M  R  R  A  E  I
     *  *  *  *  *     *  *     *     *  *     *  *

CGTGCCCGACCTGATCCGCGCGGTGTTCGAGACGGTGCCCGCCCCGTGAGCGGTCCGGAG - 1140
  V  P  D  L  I  R  A  V  F  E  T  V  P  A  P  -
  *  *     *  *     *     *     *     *     *

GAGGGGGTCAACGACGGGGCCGACATCATCTACCTCCCGCGCTGGCGGCCGGGCGGGCAC - 1200
AGGGTCGGCGCCCATCGCGGGCCGGATGCCGGCGGTCTCGGCACGTTTCGCGATCAGGTC - 1260
AGCTTCCTGCGCCTGCCGGATGGCGGGATCGACGTGCGCGCGTGCTGCGCGACCCGTTCG - 1320
AGGGCGTGTTCGTGCGCGCTTCGAGGCGGCAGCCGGTCGAGACCTACGCGCTGGTCGATC - 1380
TCTCCGCGTCCATGGCTTCCGCGGCCGGGCCGACCGGCGCGAACTGGCGGCCGGGTTCTG - 1440
   (MxaS):- M  A  S  A  A  G  P  T  G  A  N  W  R  P  G  S

CACCACGCTCGCCCGCTCGCGACGCGGATCGGCGACGGCTTCGGCCTGATCGCCTGCGAC - 1500
A  P  R  S  P  A  R  D  A  D  R  R  R  L  R  P  D  R  L  R

GACACTCTGCGCGACGACCTCACCCTCCCCCGCGACCCGCCACCGGGCCGCCGCCCAGGCC - 1560
R  H  S  A  R  R  P  H  P  P  R  D  P  P  P  G  R  R  P  G

GCCGCCGCGCGCCTCGGCGAGGCGGCCTGCGACGGACGCGGCGCCGAGGGGCTGCTGGAG - 1620
R  R  R  A  P  R  R  G  G  L  R  R  T  R  R  R  G  A  A  G

CGGCGCGCCGCCTTGCCGGGCGCCCGAAGCTGATCTTCCTCGTCTCGGATTTCCGCTGGC - 1680
A  A  R  R  L  A  G  R  P  K  L  I  F  L  V  S  D  F  R  W
   *        *                    *        *     *  *  *

CGGAGGCGCTGATCGAGGCGGTGTTCTCGGCGCTGGCGCTGCACGATGTGACCCCGGTGC - 1740
P  E  A  L  I  E  A  V  F  S  A  L  A  L  H  D  V  T  P  V
                  *           *  *              *        *

TGCTCGCCGATTCCGCCGAGGCCGAAGACCTGCCGAACTGGGGCCTCGTGGAACTCGACG - 1800
L  L  A  D  S  A  E  A  E  D  L  P  N  W  G  L  V  E  L  D
   *     *  *                    *        *  *     *  *

ATCTGGAGGGCGCCGGGCGCCGCCTCGTCTTCCTGCGCCCATCCTTGCGCCGCCGCTGGA - 1860
D  L  E  G  A  G  R  R  L  V  F  L  R  P  S  L  R  R  R  W
*     *           *  *     *           *                 *  *

TCGCCCGCGAAGCGGAGCGCGTCGAAGCTTTGCGCCGGATCTGCGCGCCCTTCGCCCGTC - 1920
I  A  R  E  A  E  R  V  E  A  L  R  R  I  C  A  P  F  A  R
   *  *     *     *        *  *  *  *  *     *

CGCCCTTTCGGCTCGCCGACCGCTTCGACGCCGAGGCGCTGAGCCGCCACCTGATGACGA - 1980
P  P  F  R  L  A  D  R  F  D  A  E  A  L  S  R  H  L  M  T

CGTGA
T  -
```

Fig. 2. The nucleotide sequence of the region between *mxaI* and *mxaA* in *M. extorquens*. Nucleotides 1–344 and the sequence downstream of 1886 have been described previously [9,20]. A possible Shine-Dalgarno site upstream of *mxaR* is underlined. The protein sequence labelled in parentheses (MxaS) represents the predicted translation product of the only open reading frame between *mxaR* and *mxaA*. The protein sequence that is in italics and underlined represents the position of the start of the putative MxaS of *P. denitrificans*. The Mg-ATP-binding Walker motifs A and B are underlined. The consensus sequence of motif A is pXXXXGXXGXGKT where p is a positively charged amino acid. The consensus sequence of motif B is RXXGXXXLfffD, where f is a hydrophobic residue. Asterisks indicate amino acids that are identical in the predicted proteins in *P. denitrificans*.