

Functional organization of a single *nif* cluster in the mesophilic archaeon *Methanosarcina mazei* strain Gö1

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Summary The mesophilic methanogenic archaeon *Methanosarcina mazei* strain Gö1 is able to utilize molecular nitrogen (N_2) as its sole nitrogen source. We have identified and characterized a single nitrogen fixation (*nif*) gene cluster in *M. mazei* Gö1 with an approximate length of 9 kbp. Sequence analysis revealed seven genes with sequence similarities to *nifH*, *nifI*₁, *nifI*₂, *nifD*, *nifK*, *nifE* and *nifN*, similar to other diazotrophic methanogens and certain bacteria such as *Clostridium acetobutylicum*, with the two *glnB*-like genes (*nifI*₁ and *nifI*₂) located between *nifH* and *nifD*. Phylogenetic analysis of deduced amino acid sequences for the nitrogenase structural genes of *M. mazei* Gö1 showed that they are most closely related to *Methanosarcina barkeri* *nif2* genes, and also closely resemble those for the corresponding *nif* products of the gram-positive bacterium *C. acetobutylicum*. Northern blot analysis and reverse transcription PCR analysis demonstrated that the *M. mazei* *nif* genes constitute an operon transcribed only under nitrogen starvation as a single 8 kb transcript. Sequence analysis revealed a palindromic sequence at the transcriptional start site in front of the *M. mazei* *nifH* gene, which may have a function in transcriptional regulation of the *nif* operon.

Keywords: *GlnB*-like proteins, *nif* genes, nitrogen fixation, nitrogen regulation.

Introduction

Biological nitrogen fixation, the enzymatic reduction of atmospheric nitrogen (N_2) to ammonia, is not limited to the bacterial domain, but is also observed in several methanogenic members of the archaeal domain. Nitrogenase, the enzyme complex of dinitrogenase and dinitrogenase reductase, is responsible for the reduction of molecular nitrogen; it is highly conserved in structure, function, and amino acid sequence across both domains (Lobo and Zinder 1992, Young 1992, Fischer 1994, Rees and Howard 1999). The dinitrogenase, which is an $\alpha_2\beta_2$ heterotetramer containing the P-cluster and the FeMo-cofactor, is encoded by *nifD* and *nifK*; the nitrogen-

ase reductase is a homodimer with a single [4Fe-4S]-cluster linking the subunits and is encoded by *nifH* (Georgiadis et al. 1992, Rees and Howard 1999 and papers cited therein). In bacteria, the genes *nifH*, *nifD* and *nifK*, which encode the molybdenum-containing nitrogenase, are typically found together in a single operon and are physically adjacent to other *nif* genes as part of a larger *nif* regulon. Downstream of *nifK*, the *nifE* and *nifN* genes, which are essential for FeMo-cofactor assembly (Dean et al. 1993), are found in a separate operon. In Archaea, genes homologous to the bacterial *nif* genes have been identified, and nitrogen fixation has been observed in several methanogenic species (Lobo and Zinder 1992, Young 1992, Bult et al. 1996, Chien and Zinder 1996, Haselkorn and Buikema 1996, Kessler et al. 1997, Smith et al. 1997). The discovery of genes homologous to *nifH*, *nifD* and *nifK* suggests that the basic mechanism of nitrogen fixation is similar in Bacteria and Archaea and predicts that most methanogenic nitrogenases contain a molybdenum-cofactor (Chien and Zinder 1996, Kessler et al. 1997). It was recently shown that, unique among the archaea, *Methanosarcina acetivorans* appears to contain all three types of nitrogenases: the molybdenum nitrogenase and two alternative nitrogenases (Galagan et al. 2002). In methanogenic archaea, the nitrogen fixation genes *nifH*, *nifD*, *nifK*, *nifE* and *nifN* are present in the same order as in bacteria (Dean and Jakobson 1992). However, in contrast, (i) methanogenic *nif* gene promoters are typical archaeal promoters, and the transcriptional apparatus is similar to that of Eucarya (Langer and Zillig 1993, Marsh et al. 1994, Langer et al. 1995, Qureshi et al. 1995, Hausner et al. 1996, Thomm 2000, Bell and Jackson 2001), (ii) the archaeal *nif* genes are present in a single operon, and (iii) all diazotrophic methanogens contain two open reading frames (ORFs) inserted between *nifH* and *nifD* that show a strong similarity to *glnB* (Sibold et al. 1991, Merrick and Edwards 1995, Arcondeguy et al. 2001, Kessler et al. 2001). Recently, this *nif* gene organization with the two *glnB*-like genes, which have been renamed *nifI*₁ and *nifI*₂ (Arcondeguy et al. 2001), has also been found in *Clostridium acetobutylicum* (Nölling et al.