GENOME ANNOUNCEMENT

Draft genomic DNA sequence of the facultatively methylotrophic bacterium *Acidomonas methanolica* type strain MB58

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Abstract

Acidomonas methanolica (former name: *Acetobacter methanolicus*) is a unique acetic acid bacterium capable of growing on methanol as a sole carbon source. We reported the draft genome sequencing of *A. methanolica* type strain MB58, showing that it contains 3270 protein-coding genes, including the genes involved in oxidation of methanol, such as *mxaFJGIRSACKL* and *hxlAB,* and oxidation of ethanol, such as *adhAB* and *adhS.*

Acidomonas methanolica MB58 (former name: *Acetobacter methanolicus* MB58) was originally isolated as a facultatively methylotrophic bacterium from a septic methanol-processing mixture in Germany (Uhlig *et al.*, 1986). *Acidomonas methanolica* is now recognized as a unique acetic acid bacterium capable of assimilating both methanol and ethanol. Thus, in addition to a methanol oxidation system found in methylotrophs, *A. methanolica* also contains an ethanol oxidase respiratory chain conserved among the acetic acid bacteria (Matsushita *et al.*, 1992). Recently, an *A. methanolica* strain CGDAM1 was isolated from a patient with chronic granulomatous disease exacerbated with cervical lymphadenopathy indicating the emerging pathogenicity of these bacteria (Chase *et al.*, 2012). Therefore, information on the *A. methanolica* genomic structure may be important for diagnostic purposes. To elucidate the genomic structure of *A. methanolica*, we have performed draft genomic DNA sequencing using *A. methanolica* type strain MB58 (NBRC 104435, ATCC 43581 and DSM 5432).

Genomic DNA sequencing and analyses were performed using a previously described method (Azuma *et al.*, 2009) with some modifications. Briefly, *A. methanolica* genomic DNA was extracted from *A. methanolica* cultured aerobically in YPGD medium at 30 °C. After DNA fragmentation and isolation (average fragment length 171 bp), 3 559 553 reads (75 bases per read) were obtained using the Illumina GAIIx sequencing system (Illumina Inc., CA). Short DNA sequences were assembled by Velvet 1.0.3 (Zerbino *et al.*, 2009). A k-mer size of 39 among different minimum-overlapping lengths gave the best results in terms of N_{50} (the median contig length), resulting in 546 contigs with total 3 690 031 bp (coverage: 90.2 x). The *A. methanolica* genome had 65.4% G + C content, which was relatively higher than that previously reported (Uhlig *et al.*, 1986). The draft genome

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sequence contained 3270 protein-coding genes, which were extracted using METAGENEANNOTATOR (MGA) (Noguchi *et al.*, 2008), and functions of the gene products were annotated by comparison with similar proteins detected by Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1997). Forty-four tRNA genes were identified by the tRNAscan-SE 1.21 program (Lowe & Eddy, 1997), and one contig (Amme_377) with an rRNA operon was identified by BLAST.

This draft genome shotgun project has been deposited as a primary project at DDBJ BioProject (the accession number: PRJDB513). The draft genome sequence of *A. methanolica* MB58 (NBRC104435, ATCC 43581 and DSM 5432) was deposited in the DDBJ/EMBL/GenBank database under the accession number BAND01000001–BAND01000546.

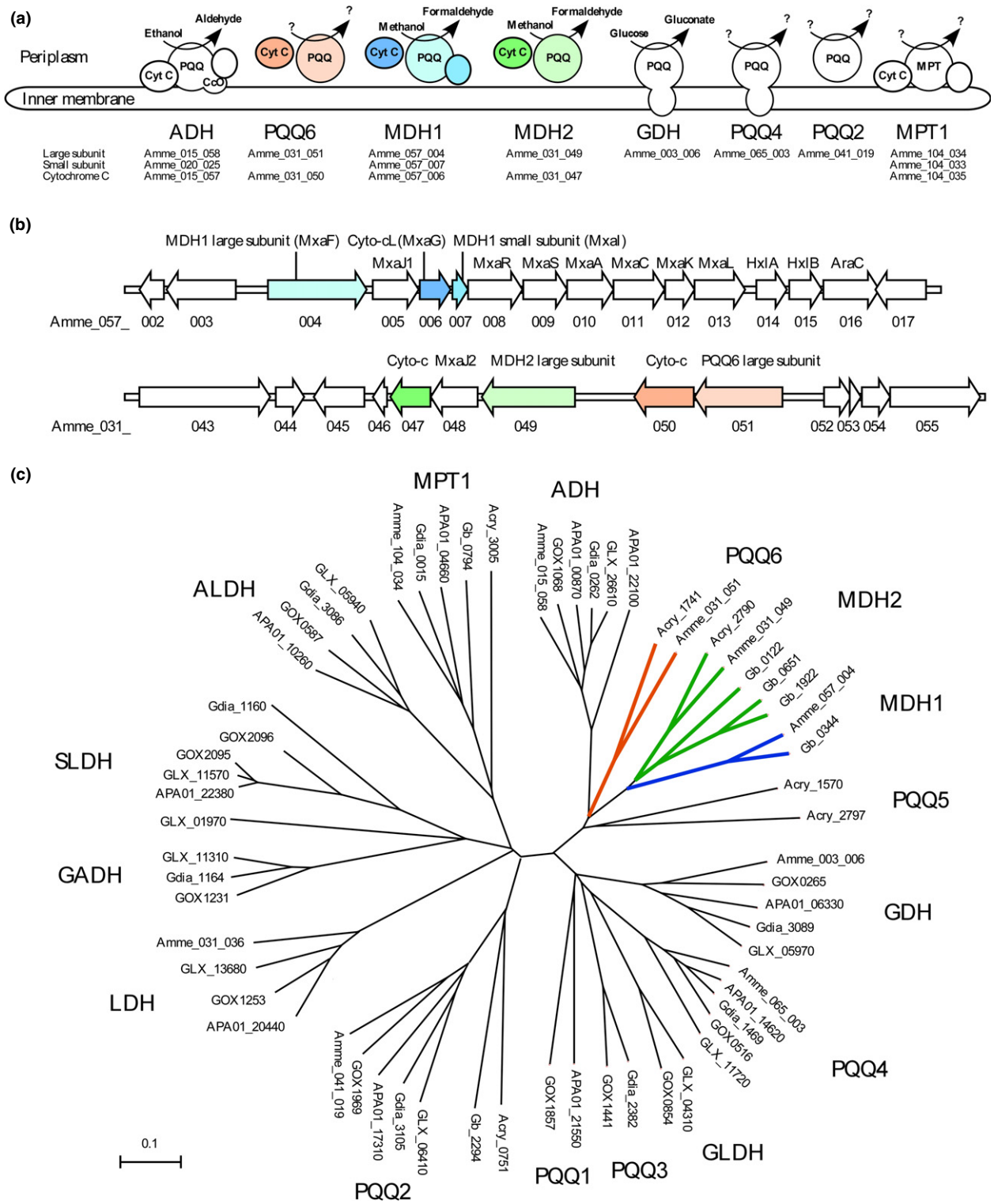
Bioinformatics analyses using BLAST (Altschul *et al.*, 1997), Pfam (Punta *et al.*, 2012) and SOSUI (Hirokawa *et al.*, 1998) demonstrated that the *A. methanolica* genome contained genes coding for eight membrane-bound primary dehydrogenases (Fig. 1a). Seven of them contained pyrroloquinoline quinone (PQQ)-binding domains, and the other one with a molybdopterin-binding domain, MPT1, showed partial similarity to the aldehyde dehydrogenase (ALDH). Of the seven dehydrogenases with the PQQ-binding domains, alcohol dehydrogenase (ADH) and methanol dehydrogenase (MDH) (named as MDH1 here) were previously shown to oxidize ethanol and methanol biochemically, respectively (Matsushita *et al.*, 1992; Frebortova *et al.*, 1998). Four PQQ-dependent enzymes, glucose dehydrogenase (GDH), another MDH (named MDH2), which demonstrated sequence similarity to MDH1, PQQ2 and PQQ4, were classified based on previously published data (Prust *et al.*, 2005). A gene encoding a PQQ-dependent dehydrogenase, which showed similarities equally to large subunits of ADH and MDH, was identified and classified into a new category, PQQ6, with a gene of *Acidiphilium cryptum* JF-5. No genes similar to PQQ-dependent glycerol dehydrogenase and FAD-dependent gluconate 2-dehydrogenase were found.

Genes coding for the MDH subunits were located in two loci (probably two operons) accompanied by 12 or more genes involved in methanol metabolism. In the first locus (Fig. 1b, top), an operon consisting of 13 genes was identified, including the ones encoding the MDH1 large subunit (MxaF), an extracellular solute-binding protein (MxaJ1), cytochrome c_L (MxaG), the MDH1 small subunit (MxaI), a transcriptional regulator for MDH (MxaR), MxaS, MxaA, MxaC, MxaK, MxaL, 6-phospho-3-hexuloisomerase (HxlB/PHI), 3-hexulose-6-phosphate synthase (HxlA/HPS) and transcriptional regulator AraC (Amaratunga *et al.*, 1997; Vuilleumier *et al.*, 2009). MxaA, MxaK and MxaL are involved in incorporation of calcium into MDH (Richardson & Anthony, 1992; Arps *et al.*, 1995), and MxaC, J and S are required for methanol oxidation (Morris *et al.*, 1995). The second locus (Fig. 1b, bottom) contained genes coding for the MDH2 large subunit, MxaJ2, cytochrome *c*, regulator protein FrmR and UDP-glucose/galactose 4-epimerase, assembled in an operon. Upstream of this operon, another operon containing the PQQ6 large subunit and cytochrome *c* was present.

HxlA/HPS and HxlB/PHI are involved in ribulose monophosphate pathway for assimilation and detoxification of formaldehyde produced from methanol by MDH (Jakobsen *et al.*, 2006) (Supporting information, Fig. S1). As no genes for glutathione-independent and glutathione-dependent formaldehyde dehydrogenases were identified in the *A. methanolica* genome, it might be the only one pathway for formaldehyde assimilation (Wilson *et al.*, 2008). As ALDH of *Corynebacterium glutamicum* was shown to be involved in the oxidation of methanol to carbon dioxide (Witthoff *et al.*, 2013), it is still possible that *A. methanolica* is able to convert formaldehyde to formate by yet uncharacterized enzyme(s), such as MPT1.

Acidomonas methanolica is the species with the ability to metabolize methanol (Suzuki *et al.*, 2009) because of the activity of MDHs and Mxa factors. Interestingly, genes, *hxlA*/*rmpA* and *hxlB*/*rmpB*, encoding HxlA/HPS and HxlB/PHI, respectively, were not found in any other genome sequences of acetic acid bacteria deposited in public databases, but many species of Gram-positive *Microbacteriaceae* and *Micrococcaceae* families contain the homologues. Acquisition of the genes, *hxlA*/*rmpA* and *hxlB*/*rmpB*, might be a key point to survive efficiently with methanol as a sole carbon source for *A. methanolica*. In the meantime, a member of *Acetobacteraceae* family, *Granulibacter bethesdensis*, like *A. methanolica*, causes a

Fig. 1. Primary dehydrogenases of *Acidomonas methanolica* and other acetic acid bacteria. (a) Schematic presentation of primary dehydrogenases predicted from genes identified in *A. methanolica* MB58. Incorporated cofactors, PQQ and MPT domains were identified in the large subunits (Matsushita *et al.*, 1992). IDs of the genes coding for the enzymes or the subunits are indicated under each enzyme name. (b) Structures of the methanol metabolism-related genes in the two loci. Top and bottom panels indicate parts of the contig number 57 (Amme_057) and 31 (Amme_031), respectively. (c) Phylogram of the primary dehydrogenases (or their large subunits) identified in genomes of *Acetobacter pasteurianus* IFO 3283-01 (Azuma *et al.*, 2009), *Gluconacetobacter diazotrophicus* PAl 5 (Bertalan *et al.*, 2009), *G. xylinus* NBRC 3288 (Ogino *et al.*, 2011), *Gluconobacter oxydans* 621H (Prust *et al.*, 2005), *Acidiphilium cryptum* JF-5 (GENBANK: from NC-009467 to NC-009474 and NC-009484), *Granulibacter bethesdensis* CGDNIH1 (Greenberg *et al.*, 2007) and *A. methanolica* MB58 in this report.



chronic granulomatous disease, grows on methanol as a sole carbon source and contains most of all the methanol oxidation-related genes (Greenberg *et al.*, 2006, 2007). However, genes coding for HxlA/HPS, HxlB/PHI and formaldehyde dehydrogenases were not identified in *G. bethesdensis*, and it is currently unknown how this bacterium utilizes methanol and detoxifies harmful formaldehyde.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Methanol oxidization and formaldehyde assimilation.