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NOTES AND SHORT COMMUNICATIONS

Genome Characteristics of a Novel Type I Methanotroph (Sn10-6) Isolated from a Flooded Indian Rice Field

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Abstract Flooded rice fields are important sources of atmospheric methane. Aerobic methanotrophs living in the vicinity of rice roots oxidize methane and act as environmental filters. Here, we present genome characteristics of a gammaproteobacterial methanotroph, isolate Sn10-6, which was isolated from a rice rhizosphere of a flooded field in India. Sn10-6 has been identified as a member of a putative novel genus and species within the family *Methylococcaceae* (Type I methanotrophs). The draft genome of Sn10-6 showed pathways for the following: methane oxidation, formaldehyde assimilation (RuMP), nitrogen fixation, conversion of nitrite to nitrous oxide, and other interesting genes including the ones responsible for survival in the rhizosphere environment. The majority of genes found in this genome were most similar to *Methylovulum miyakonese* which is a forest isolate. This draft genome provided insight into the physiology, ecology, and phylogeny of this gammaproteobacterial methanotroph.

Keywords Methanotrophs · Flooded rice fields · Draft genome

Introduction

Flooded rice fields are important sources of methane, which is the second most important greenhouse gas responsible for global warming [1]. Aerobic methanotrophs dwelling in the vicinity of rice roots oxidize methane and thus play an important role in methane mitigation [2]. Classical methanotrophs are classified into two groups (Type I and Type II) methanotrophs and belong to Gammaproteobacteria and Alphaproteobacteria, respectively [3]. Recently, methanotrophs have also been reported from Verrucomicrobial phylum; however, these species are mostly present in acidic habitats [4, 5]. Though Type I methanotrophs play an important role in methane oxidation in the rhizospheric environments of flooded rice fields, very few members have been isolated and characterized and described from such habitats. To the best of our knowledge, only two species of gammaproteobacterial methanotrophs (Type I) have been formally described from rice field environments [6, 7]. These are *Methylogaea oryzae* which was isolated from rice fields in Uruguay [8] and *Methylomonas koyamae* which was isolated from Japanese rice fields [9]. Genome information for either of these species is not yet available. In our recent work, we enriched, isolated and characterized methanotrophs from a rice rhizosphere environment in India (Pandit, P., Rahalkar, M. and others, accepted). Isolate Sn10-6 was isolated from a dilution series liquid enrichment (10^{-6} dilution) of a rice rhizospheric soil sample incubated in presence of methane, air, and nitrogen. The sample was collected from a flooded rice field in Western India. Blast analysis of the 16S rRNA gene of Sn10-6 (KP793700.1) showed only 90–93.5 % similarity with Type I methanotrophic genera and hence represented a member of a putative novel genus and species within *Methylococcaceae*. Using EzTaxon server (http://www.ezbiocloud.net/eztaxon/), the closest neighbor was found to be *Methylosarcina lacus* LW14^T (93.5 %). After phylogenetic analysis of 16S rRNA genes, Sn10-6 belonged to a distinct branch next to *Methylovulum-Methylosoma* clade.

We sequenced the genome of Sn10-6 in order to get a clearer idea of its taxonomy, physiology, and ecology. Moreover, Sn10-6 is one of the first gammaproteobacterial

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methanotroph recovered from rice rhizospheres of flooded paddy fields in a tropical country like India. Any information about the metabolism of strain Sn10-6 that can be obtained from analysis of the genome will also help us understand its role in biogeochemical cycling in flooded rice field ecosystems. We additionally investigated the genome for genes which might be helping the organism to survive in the rhizosphere environment. Thus, the main aim of this paper was to study the salient characteristics of the genome of a novel gammaproteobacterial (Type I) methanotroph which was isolated from a flooded rice field in India.

After DNA extraction and confirmation of the 16S rRNA gene sequence, whole genome sequencing of Sn10-6 was done using the Ion Torrent PGM (200 bp) with the 316[TM] sequencing chip. We performed de novo assembly using the MIRA 4.0.5 assembler [10] which generated 389 contigs. It was revealed that genome size of Sn10-6 is 4.58 Mb with a G+C content of 43.9 % (Table 1). The longest contig was 91, 582 bp with N50 of 19,087 bp and N90 4856 bp. This data was then submitted to the Rapid Annotation using Subsystem Technology (RAST server) [11, 12] and NCBI for annotation. Annotation by NCBI revealed presence of 3413 proteins. We additionally used BlastKOALA (http://www.kegg.jp/blastkoala/) for prediction of KEGG pathways. Single copy of SSU rRNA and LSU rRNA were found in the genome along with 43 tRNAs (Table 1). Using the RAST server, the closest genome match was *Methylobacter tundripaludum* (genome id: 697282.3) with a score of 524.

The genome of strain Sn10-6 showed all the necessary machinery required for methane metabolism (Fig. 1). Single copy of the *pmo* CAB gene cluster encoding particulate methane monooxygenase enzyme (pMMO), enzyme responsible for conversion of methane to methanol, the first step in the methane oxidation pathway, was found. Additionally, many of the methanotrophs also show a homologue of *pmo* CAB, denoted as *pmx* ABC operon [13]. Genes for *pmx* ABC and the soluble methane monooxygenase (sMMO) were both missing from the genome. sMMO is present only in a few genera within Type I methanotrophs and is mostly present in Type X and Type II methanotrophs [3]. pMMO genes in Sn10-6 were most similar to genes in *M. miyakonese* and
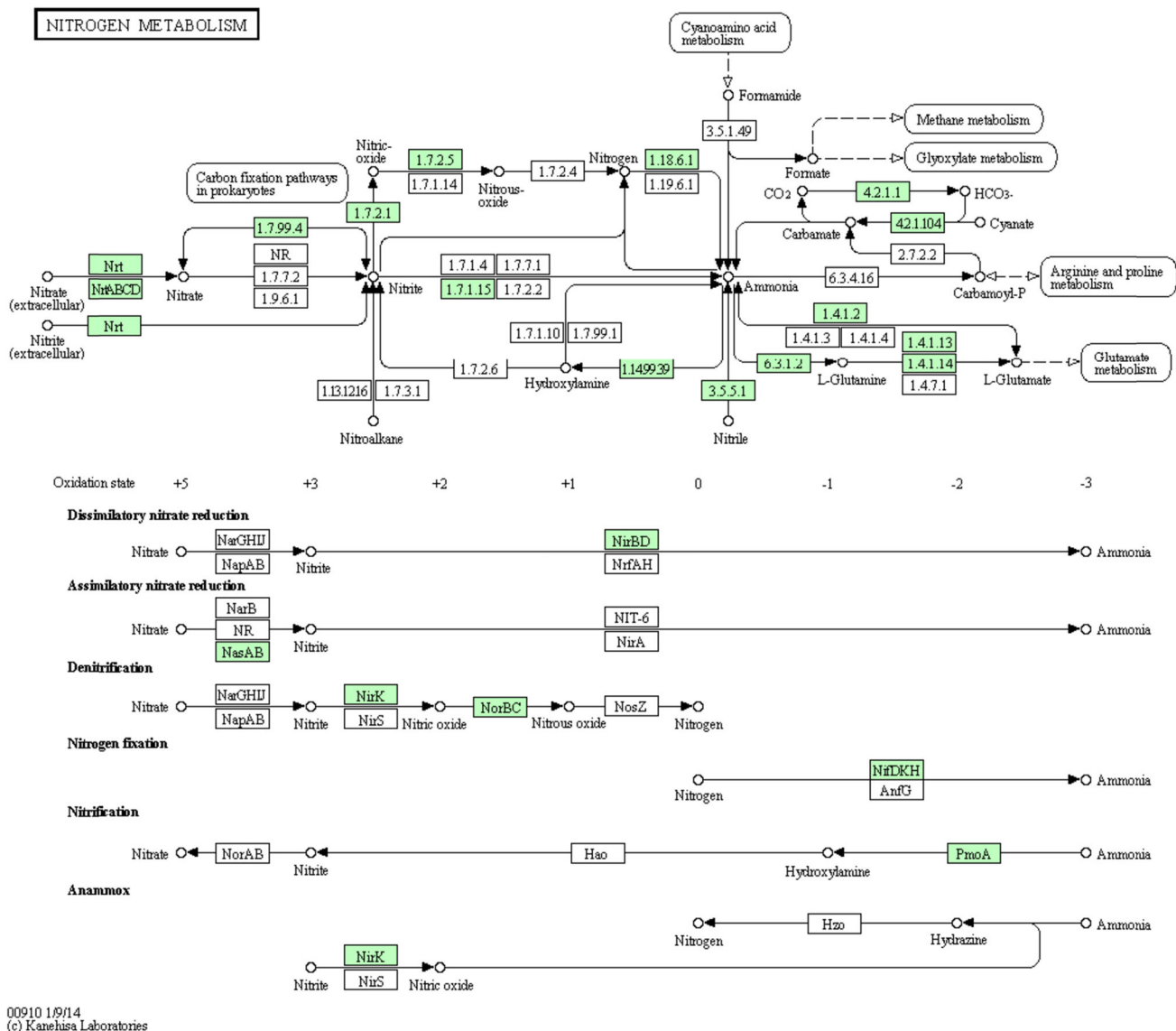
M. tundripaludum as revealed by blastx analysis (Table 2). The second step in the pathway is conversion of methanol to formaldehyde by the enzyme methanol dehydrogenase. Genes coding for all subunits of methanol dehydrogenase showed highest homology to those from *M. miyakonese* (Table 2). Next step in the pathway is oxidation of formaldehyde to formate. In Sn10-6, the formaldehyde oxidation occurs probably by the tetrahydromethanopterin (H$_4$MPT) pathway. Various enzymes for the H$_4$MPT pathway were detected which included 5,10-methylenetetrahydrofolate reductase (EC 1.5.1.20), methylene tetrahydromethanopterin dehydrogenase (EC 1.5.99.9), formate-tetrahydrofolate ligase (EC 6.3.4.3), formiminotetrahydrofolate cyclodeaminase (EC 4.3.1.4) and N(5), N(10)-methenyltetrahydromethanopterin cyclohydrolase (EC 3.5.4.27). Tetrahydromethanopterin pathway has been discovered as one of the ancient and major pathways for formaldehyde oxidation [14] and is found to be present in some of the methanotrophic and methylotrophic genome sequences described recently [15]. The last step in the methane oxidation pathway is encoded by formate dehydrogenase enzyme. Formate dehydrogenase in Sn10-6 was most similar to that of *Methylovulum miyakonese*. Formaldehyde acts as the central intermediate in methanotrophs and is used for assimilation. In Type I methanotrophs, formaldehyde assimilation normally takes place via ribulose mono phosphate (RuMP) pathway [3]. Genes for RuMP pathway were found in Sn10-6 genome confirming that it was a typical Type I methanotroph (Fig. 1). Even though methanotrophs can use only C1 compounds (methane and in some cases methanol) as their carbon and energy source, genes for glycolysis, tricarboxylic acid (TCA) cycle are present in many species [13]. Similarly, in Sn10-6, genes for glycolysis, pentose phosphate pathway, TCA cycle were present. Similarly, genes encoding acetate kinase and acetyl Co-A synthetase were also detected. Many of the genes in the carbon metabolism were most related to the genes of *M. miyakonese*.

We detected genes that coded for proteins involved in nitrogen metabolism which included genes for assimilatory nitrate reduction, nitrogen fixation, nitrite to nitrous oxide conversion, and urea or ammonium utilization (Fig. 2). Although dissimilatory nitrate reduction genes were absent in the genome, Sn10-6 shows genes required for conversion of nitrite to nitrous oxide. Genes of this pathway have been detected in *M. miyakonese*, *Methylomicrobium alcaliphilum* [15] and *Methylomonas methanica* MC09 [16] (Table 2). Nitrogen fixation can be an important process for bacteria living in rhizospheres with respect to their interactions with plants. The metabolic pathway for the conversion of nitrite to nitrous oxide in Sn10-6 was also noteworthy. Most of the nitrogen metabolism genes were also most related to *M. miyakonese* (*nifH* genes, nitric oxide reductase, and urease) and a few to *M. tundripaludum* (assimilatory nitrate reductase).

Table 1 Genome features of strain Sn10-6 (LAJX00000000.1)

Attributes	Values
Genome size	4.58 Mbp
Contigs	389
G+C content	43.9 %
Proteins	3413
tRNA	43
rRNA	5 (one copy of 16S and 23S rRNA)
ncRNA	1

Fig. 1 Genes related to methane metabolism as detected in Sn10-6 (shown in *green*). This figure was generated by submitting the assembled contigs (amino acids) to BlastKOALA (http://www.kegg.jp/blastkoala/) for prediction of KEGG pathways

Table 2 Blastx analysis of genes involved in carbon and nitrogen metabolic pathways

Name of the enzyme/gene	Nearest neighbor using Blastx with percentage similarity
Particulate methane monooxygenase C (*pmoC*)	*Methylobacter tundripaludum* (91)
Particulate methane monooxygenase A (*pmoA*)	*Methylovulum miyakonese* (92)
Particulate methane monooxygenase B (*pmoB*)	*Methylobacter tundripaludum*, *Methylovulum miyakonese* (84)
Methanol dehydrogenase large subunit protein (EC 1.1.99.8) *mxaF*	*Methylovulum miyakonese* (92)
Formate dehydrogenase (alpha subunit)	*Methylovulum miyakonese* (88)
Formylmethanofuran dehydrogenase (subunit A, B)	*Methylovulum miyakonese* (91, 76)
Formylmethanofuran H$_4$MPT formyl transferase	*Methylobacter luteus* (91)
N(5),N(10)-methenyltetrahydromethanopterin cyclohydrolase	*Methylovulum miyakonese* (82)
6-Phospho-3-hexuloisomerase	*Methylovulum miyakonese* (84)
Nitrogenase (*nifH*) gene	*Methylovulum miyakonese* (97)
Assimilatory nitrate reductase	*Methylobacter tundripaludum* (74)
Nitric oxide reductase	*Methylovulum miyakonese* (86)
Urease (alpha)	*Methylovulum miyakonese* (88)

Annotation generated by RAST analysis was used for blastx analysis

Fig. 2 Genes related to nitrogen metabolism as detected in Sn10-6 (shown in *green*). This figure was generated by submitting the assembled contigs (amino acids) to BlastKOALA (http://www.kegg.jp/blastkoala/) for prediction of KEGG pathways

In addition, genes coding for type IV pili, flagella, and chemotaxis were also detected. These genes could especially be important for the motility of the organism according to the changing methane and oxygen gradients within the rhizosphere microenvironment. We also found another interesting genes, i.e., MazF/MazE genes which are responsible for toxin-antitoxin genes involved in programmed cell death occurring after nutrient starvation [17, 18]. After blast analysis, MazF (toxin) genes in Sn10-6 were found most related to that of *M. miyakonese*. None of the other gammaproteobacterial methanotrophs (other than *M. miyakonese* and Sn10-6) have been reported to have MazE/MazF, and hence, their function in these two methanotrophs warrants further investigation. Nutrient starvation might be a common situation for the rice field methanotrophs during the dry periods of the year [19], and

therefore, these genes would be worth of further exploration. Apart from these genes, we also detected genes for carotenoid pigment, flagellar apparatus, and rod shape which explain the morphology of this pale pink pigmented motile methanotroph.

Thus, studying the draft genome of Sn10-6 gave us important insights into the metabolism and helped us to understand its lifestyle as a rice rhizosphere associated gammaproteobacterial methanotroph living in paddy fields of a tropical country.

Nucleotide sequence accession number The draft genome sequence of *Methylococcaceae* bacterium strain Sn10-6 is deposited in the GenBank database under the accession number LAJX00000000.1 and the bioproject is registered as PRJNA278928.

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