ISSN 0026-2617, Microbiology, 2007, Vol. 76, No. 1, pp. 118–121. © Pleiades Publishing, Ltd., 2007.
Original Russian Text © I.S. Tsyrenzhapova, B.Ts. Eshinimaev, V.N. Khmelenina, G.A. Osipov, Yu.A. Trotsenko, 2007, published in Mikrobiologiya, 2007, Vol. 76, No. 1, pp. 132–135.

SHORT COMMUNICATIONS

A New Thermotolerant Aerobic Methanotroph from a Thermal Spring in Buryatia

I. S. Tsyrenzhapova[a,b], B. Ts. Eshinimaev[a,b], V. N. Khmelenina[a], G. A. Osipov[c], and Yu. A. Trotsenko[a,b,1]

[a] *Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia*
[b] *Pushchino State University, pr. Nauki 3, Pushchino, Moscow oblast, 142290 Russia*
[c] *Academic Group of Russian Academy of Medical Sciences, Moscow, Russia*

Received May 17, 2006

DOI: 10.1134/S0026261707010171

Aerobic methanotrophs belong to a specific group of bacteria that utilize methane as a source of carbon and energy. Thermophilic and thermotolerant methanotrophs are of great interest to researchers due to their mechanisms of thermoadaptation and their potential biotechnological applications [1, 2]. For a long time, the range of collection thermophilic and thermotolerant methanotrophs has been limited to the genus *Methylococcus* [3]. The intense study of thermal springs in Hungary and Japan by methods of general microbiology and molecular biology, as well as the revision of the genus *Methylococcus*, have led to the isolation of moderately thermophilic methanotrophs capable of growth at 37–62°C and description of two new genera—*Methylocaldum* [4, 5] and *Methylothermus* [2, 6]. The currently known thermophilic methanotrophs are the species *Methylothermus thermalis* and *Methylocaldum szegediense*, and the known thermotolerant methanotrophs are the species *Methylococcus capsulatus, Methylocaldum gracile*, and *Methylocaldum tepidum.* These bacteria have type I intracytoplasmic membranes (ICMs) and possess either a ribulose monophosphate (RuMP) pathway alone (the genus *Methylothermus*) or two additional minor carbon assimilation pathways with serine and ribulose bisphosphate as specific intermediates (the genera *Methylococcus* and *Methylocaldum*). The diversity of methanotrophs living in high-temperature ecosystems is obviously not limited to the aforementioned taxa. Indeed, this article describes a new thermotolerant methanotroph isolated from a thermal spring in the Transbaikal region.

The radioisotopic study [7] of bottom sediment samples (courtesy of B.B. Namsaraev from the Institute of General and Experimental Biology, Russian Academy of Sciences, Siberian Division, Ulan-Ude, Russia) collected in thermal springs with water temperatures of 48–55°C (Alla, Kuchiger, Sukhaya, and Seya) showed that the methane oxidation rate in these sediment samples reaches 4 nmol ml^{-1} day^{-1}. The enrichment cultures N3, N6, Suh1, and Suh2 isolated from these samples at 45°C (this temperature is close to that in situ) represented closely associated methanotrophs and heterotrophic satellite bacteria. The electron microscopic examination of these cultures showed that methanotrophic cells are gram-negative vibrioids 0.3–0.4 × 0.9–1.1 μm in size, with peripheral type II ICMs.

A pure culture of Se48 was isolated from the mud sample collected in the Seya spring. The isolation was carried out by the method of accelerated subculturing at 45°C using a liquid P medium [8] with half of its normal mineral content. The study of the geno- and phenotypic properties of the isolate by the methods described earlier [1, 2] showed that, when grown on solid media for 7–10 days, it produces round, convex, nonpigmented colonies 2–3 mm in size. Strain Se48 represents gram-negative vibrioids 0.6–1.2 × 1.2–1.7 μm in size, enclosed in a polysaccharide capsule. The cells contain type II ICMs and poly-β-hydroxybutyrate granules. The DNA isolated from strain Se48 was found to contain the *nifH* gene, which encodes nitrogenase.

The optimal and maximal growth temperatures of strain Se48 are 37 and 53°C, respectively. Therefore, strain Se48 may be considered to be a thermotolerant methanotroph. It should be noted that strain Se48 differs from other type II methanotrophs in that the former has a higher maximal growth temperature. This fact is in agreement with the higher temperature of the spring from which the strain was isolated.

An enzymatic analysis showed that strain Se48 assimilates carbon via the serine pathway containing no isocitrate lyase. The low activity of the 2-oxoglutarate dehydrogenase complex suggests the presence of the complete tricarboxylic acid cycle, which mainly performs a biosynthetic function. Gluconeogenesis proceeds with the involvement of pyrophosphate-depen-

---

[1] Corresponding author; e-mail: trotsenko@ibpm.pushchino.ru

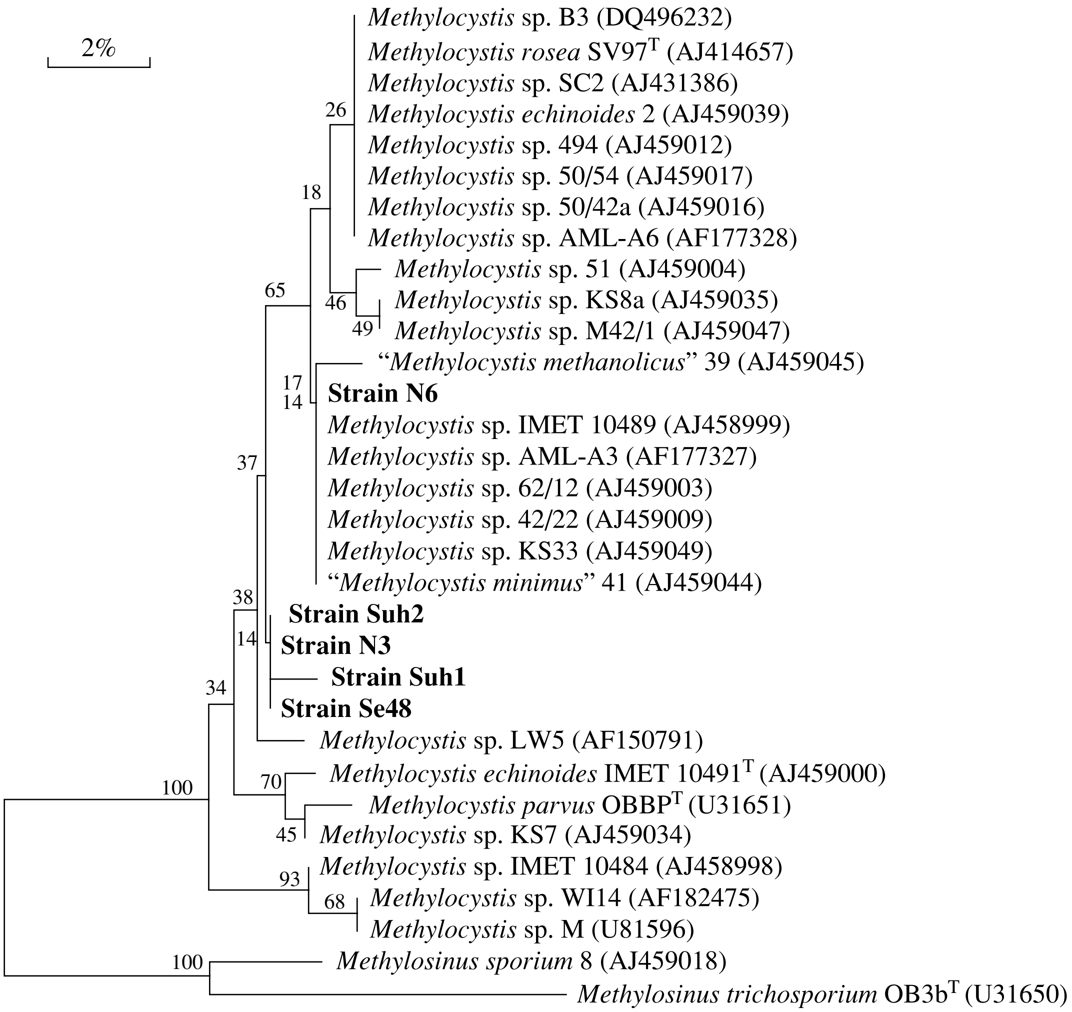
Comparative properties of strain Se48 and some species of the genus *Methylocystis*

Parameter	*M. parvus* [8, 9]	*M. echinoides* [8, 9]	*M. rosea* [10]	Strain Se48
Cell shape	Pyriform vibrioides	Vibrioides	Rods	Vibrioides
Cell size, μm	0.5–0.7 × 0.8–2.0	0.5–0.7 × 0.8–1.2	0.8–1.1 × 1.1–2.5	0.6–1 × 1.2–1.7
Motility	–	–	–	–
Capsule	+	–	ND	+
Colony color	White	White to rose	Rose–red	White
C_1-assimilation pathway	Serine-type	Serine-type	Serine-type	Serine-type
pMMO	+	+	+	+
sMMO	ND	–	–	–
Nitrogen fixation	+	+	+	+
Phospholipids	PC, PEA, PMMEA	PC, PEA, PDMEA	ND	PC, PEA, CL, PS
Major fatty acids	18:1ω7c 18:1ω8c	18:1ω7c 18:1ω8c	18:1ω7c 18:1ω8c	18:1ω7c 18:1ω9c
pH range	6.0–8.5	6.0–8.0	5.5–9.0	5.0–7.5
pH optimum	6.5–7.5	6.5–7.5	ND	5.5–6.8
Growth temperature range, °C	15–37	15–30	5–37	15–53
Growth temperature optimum, °C	28–30	27	27	37
G+C content, mol %	62.5	63.1	62	62

Notes: "+" and "–" stand for, respectively, the presence and absence of a given trait. ND stands for "not determined." pMMO, particulate methane monooxygenase; sMMO, soluble methane monooxygenase; PC, phosphatidylcholine; PS, phosphatidylserine; CL, cardiolipin; PEA, phosphatidylethanolamine; PMMEA, phosphatidylmonomethylethanolamine; PDMEA, phosphatidyldimethylethanolamine.

dent 6-phosphofructokinase. NH_4^+ is assimilated via the glutamate cycle, but not through the reductive amination of 2-oxoglutarate or pyruvate.

Strain Se48 differs from mesophilic type II methanotrophs in the predominance of monounsaturated octadecenoic acids, $C_{18:1\omega9c}$ (60%) and $C_{18:1\omega7c}$ (33%), and the absence of $C_{18:1\omega8c}$, which is the marker fatty acid of the genera *Methylosinus/Methylocystis* (see table). On the basis of a comparison of the deduced amino acid sequences of the *pmoA* gene made with the aid of the BioEdit program (version 7.0.1), strain Se48 was assigned to the genus *Methylocystis* (see figure). Strain Se48, with other methanotrophs from the N3, Suh1, and Suh2 enrichment cultures, forms a separate cluster on the PmoA-based phylogenetic tree of type II methanotrophs (*Alphaproteobacteria*), showing the highest degree of homology (98%) to the translated amino acid sequences of *Methylocystis parvus*.



Dendrogram constructed on the basis of a comparison of the translated amino acid sequences of the *pmoA* gene of methanotrophs isolated from the thermal springs of Buryatia and other type II methanotrophs. The dendrogram was constructed with the aid of Treecon for Windows (version 1.3b).

Thus, the study of the microbial communities of the thermal springs of Buryatia demonstrates the presence of methanotrophs in these ecosystems. The ability of strain Se48 to grow at 53°C suggests that it has a wider range of growth temperatures than other methanotrophs of the genus *Methylocystis* (table). This circumstance offers great scope for a comparative study of the structural and functional fundamentals of thermoadaptation in type I and type II methanotrophs.

This work was supported by the Russian Foundation for Basic Research, grant no. 05-04-49515, and by the Ministry of Education and Science of the Russian Federation, grant RNP 2.1.1.2671.

REFERENCES

1. Eshinimaev, B.Ts., Medvedkova, K.A., Khmelenina, V.N., Suzina, N.E., Osipov, G.A., Lysenko, A.M., and Trotsenko, Yu.A., New Thermophilic Methanotrophs of the Genus *Methylocaldum, Mikrobiologiya*, 2004, vol. 73, no. 4, pp. 530–539.
2. Tsubota, J., Eshinimaev, B.T., Khmelenina, V.N., and Trotsenko, Y.A., *Methylothermus thermalis* gen. nov., sp. nov., a Novel Moderately Thermophilic Obligate Methanotroph from a Hot Spring in Japan, *Int. J. Syst. Evol. Microbiol.*, 2005, vol. 55, pp. 1877–1884.
3. Malashenko, Yu.R., Romanovskaya, V.A., Bogachenko, V.N., and Shved, A.D., Thermophilic and Thermotolerant Bacteria Assimilating Methane, *Mikrobiologiya*, 1975, vol. 44, no. 5, pp. 855–862.
4. Bodrossy, L., Holmes, E.M., Holmes, A.J., Kovacs, K.L., and Murrell, J.C., Analysis of 16S rRNA and Methane Monooxygenase Gene Sequences Reveals a Novel Group of Thermotolerant and Thermophilic Methanotrophs, *Methylocaldum* gen. nov., *Arch. Microbiol.*, 1997, vol. 168, no. 6, pp. 493–503.
5. Romanovskaya, V.A., Rokitko, P.V., Shilin, S.O., and Malashenko, Yu.R., Reclassification of Thermophilic Methane-oxidizing Bacteria by the Sequence Analysis

of 16S rRNA Genes, *Mikrobiol. Zh.*, 2006, vol. 68, no. 1, pp. 3–10.

6. Bodrossy, L., Kovacs, K.L., McDonald, I.R., and Murrell, J.C., A Novel Thermophilic Methane-Oxidizing γ-*Proteobacterium*, *FEMS Microbiol. Letts.*, 1999, vol. 170, no. 2, pp. 335–341.
7. Sokolov, A.P. and Trotsenko, Y.A., Methane Consumption in (Hyper)saline Habitats of Crimea (Ukraine), *FEMS Microbiol. Ecol.*, 1995, vol. 18, pp. 299–304.
8. Gal'chenko, V.F., *Metanotrofnye bakterii* (Methanotrophic Bacteria), Moscow: GEOS, 2001.
9. Bowman, J.P., Sly, L.I., Nichols, P.D., and Hayward, A.C., Revised Taxonomy of the Methanotrophs: Description of *Methylobacter* gen. nov., Emendation of *Methylococcus*, Validation of *Methylosinus* and *Methylocystis* Species, and a Proposal That the Family *Methylococcaceae* Includes Only the Group I Methanotrophs, *Int. J. Syst. Bacteriol.*, 1993, vol. 43, pp. 735–753.
10. Wartiainen, I., Hestnes, A.G., McDonald, I.R., and Svenning, M.M., *Methylocystis rosea* sp. nov., a Novel Methanotrophic Bacterium from Arctic Wetland Soil, Svalbard, Norway (78°N), *Int. J. Syst. Evol. Microbiol.*, 2006, vol. 56, pp. 541–547.