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SHORT COMMUNICATIONS

First Isolation of a Type II Methanotroph from a Soda Lake

B. Ts. Eshinimaev[a], **V. N. Khmelenina**[a], **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*

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Soda lakes are alkaline environments, often with high mineralization. In microbial communities of alkaline and saline reservoirs, members of the genera *Methylomicrobium* and *Methylobacter*, type I methanotrophs using the most efficient ribulose monophosphate (RMF) pathway for assimilation of C_1 compounds, are the major aerobic methanotrophs [1]. This is probably the result of selective pressure of high alkalinity and salinity, which require additional expenditure of energy to maintain ionic homeostasis. Moreover, in the course of enrichment and isolation of pure cultures, enhanced content of mineral salts favored the most adapted forms. Since no type II methanotrophs have been isolated by approaches that considered in situ conditions and the physiological differences between two main methanotrophic groups, their existence in soda lakes seemed doubtful. However, recent molecular biological studies of soda lakes of Mongolia, Southeastern Transbaikalia, and the United States suggested the presence of type II methanotrophs (with the serine pathway of carbon assimilation) [2–4]. The goal of the present work was therefore isolation and primary characterization of a morphotype II methanotroph from a Southeastern Transbaikalia soda lake.

Bottom sediments were collected from the Maloe Guzhirnoe soda lake (Southeastern Transbaikalia) with low mineralization and pH 9.7. Their 1.5-month incubation under stationary conditions in a fourfold diluted P mineral medium [5] with 12.5 mM $NaHCO_3$ resulted in an enrichment culture with predominating cells similar to type II methanotrophs. Strain B3 was isolated by serial dilutions and plating on agarized medium. Its growth in liquid media was unstable; stirring often resulted in cell lysis.

The effect of medium composition on $^{14}CH_4$ consumption was determined using fourfold diluted P medium (0.25 P) with 12.5 mM $NaHCO_3$; pH 9.5–9.7. In the experiment, the concentrations of phosphates, $CaCl_2$, $MgSO_4$, KNO_3, and trace elements were increased fourfold. Media P and 0.25 P were used as controls. The biomass was grown of 0.25 P agarized medium, resuspended in 1 ml of the medium (OD_{600} = 0.5), supplemented with $^{14}CH_4$ (10 µCi, Izotop, Russia), and incubated for 24 h at 26°C. Under alkaline conditions, $CaCl_2$ and KNO_3 inhibited assimilation, while $MgSO_4$ stimulated methane consumption. Increased carbonate content (up to 50–100 mM) also had an inhibitory effect. No inhibition was observed under neutral conditions. Further studies of the physiological, biochemical, and chemotaxonomic characteristics of strain B3 were carried out as described previously [6].

The cells of strain B3 are nonmotile gram-negative ovoids; the location of the intracytoplasmic membranes (ICM) in parallel to the cell wall was characteristic of type II methanotrophs. On agarized media at neutral pH, cream-colored colonies (1–2 mm in diameter) with even edges and smooth surfaces are formed after one to two weeks of incubation. On media with carbonates, the colonies become light brown in color. The isolate does not grow with formate or C_n compounds as carbon and energy sources; it is incapable of autotrophic growth. Nitrate and ammonium can be used as nitrogen sources.

Strain B3 grows within the temperature range from 8 to 37°C, with the optimum at 26°C. The pH growth range is 6.0 to 9.5, with the optimum at pH 7.4. The highest growth rate was detected at 0.2% NaCl; no growth occurred at NaCl concentrations exceeding 0.5%. In the fatty acid profile, 9-octadecenoic acid predominates (95% of the total fatty acids). The 10- and 11-*cis*-octadecenoic acids characteristic of *Methylocystis/Methylosinus* and *Methylocella/Methylocapsa* were not detected (table). The fatty acid profile of strain B3 was determined in the cells grown on agarized (1.5%) 0.25 P medium at pH 9.5–9.7. Q_{10} is the major ubiquinone.

Strain B3 contains only the membrane-bound methane monooxygenase (MMO). Neither activity of the soluble MMO, nor the presence of the *mmoX* gene encoding this enzyme was revealed. Activity of hydroxypyruvate reductase (244 nmol min^{-1} (mg protein)$^{-1}$ and serine-glyoxylate aminotransferase (81 nmol min^{-1} (mg protein)$^{-1}$, the indicator enzymes of the serine

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

Characteristics of strain B3 and other members of the genus *Methylocystis*

Characteristics	Strain B3	*M. echinoides* [10]	*M. parva* [10]	*M. rosea* [7]	*M. hirsuta* [8]	*M. heyeri* [9]
Cell morphology	Ovoids	Ovoids	Ovoids	Rods	Ovoids	Rods
Size, μm	1.5 × 2.0	0.5–0.7 × 0.8–1.2	0.3–0.5 × 0.5–1.5	1.1–2.5 × 0.8–1.1	0.3–0.6 × 0.7–1.0	0.8–1.2 × 1.4–4.0
Pigmentatio	Cream-colored	Cream-colored	Light brown	Pink	Cream-colored	Cream-colored
pH range	6.0–9.5	6.0–8.0	5.0–9.0	5.0–9.0	4.0–9.0	4.4–7.5
optimum	7.4	6.5–7.5			7.0	5.8–6.2
Carbonate range	0–0.05	ND	ND	ND	ND	ND
optimum, M	0.012					
NaCl range	0–0.5	0–1	0–2	0.01–1	ND	0–0.5
optimum, %	0.2					
Temperature of growth, °C	8–37	15–30	Up to 45	5–37	Up to 37	5–30
optimum, °C	26	27–30	28	27	30	25
Predominant fatty acids	18:1ω9c	18:1ω7c 18:1ω8c	18:1ω8c	18:1ω8 18:1ω7 16:1ω7	ND	16:1ω8c 18:1ω8c
Ubiquinone	Q_{10}	Q_8	ND	ND	ND	ND
G+C, mol %	60.5	63	64–67	62	ND	61–62

Note: ND, not determined.

pathway, were detected in the cell-free extracts. No activity was detected of the key enzymes of the RMP and RBP cycles, hexulose phosphate synthase and ribulose bisphosphate carboxylase (RubisCO). Activity of α-ketoglutarate dehydrogenase (11 nmol min^{-1} mg $protein^{-1}$) indicates the existence of a closed TCA cycle. Strain B3 assimilates nitrogen via reductive deamination of α-ketoglutarate and via the glutamate cycle.

The DNA G+C base content of the strain is 60.5 mol %. Comparison of *pmoA* translated amino acid sequences with those represented in GenBank revealed the closest similarity of strain B3 and methanotrophs of the genus *Methylocystis* (Fig. 1). According to 16S rRNA gene sequences, the strain is most closely related to the recently described *Methylocystis rosea* SV97 (99.6%) [7] and *Methylocystis hirsuta* (99%) [8] (Fig. 2). High similarity of 16S rRNA gene sequences and the differences in the fatty acid spectra do not enable precise taxonomic identification of strain B3; DNA–DNA hybridization and further physiological and biochemical investigation are required for this purpose.

Thus, strain B3 is the first and presently the only type II methanotroph isolated from soda lakes. Although its optimal growth occurs under the conditions different from the hydrochemical parameters of a soda lake, growth in a relatively broad pH range is possible. Other components of the microbial community contribute possibly to methanotroph's survival in this

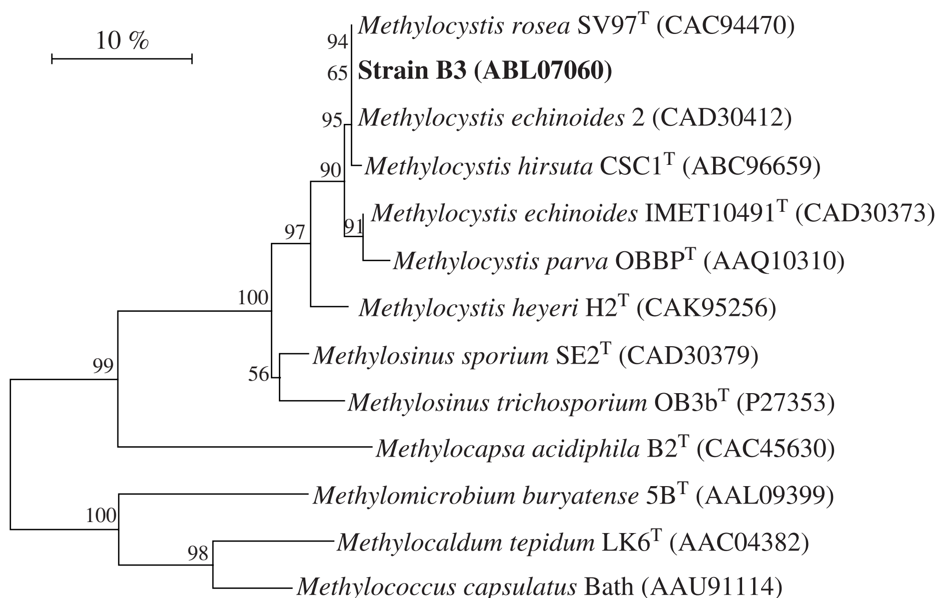

Fig. 1. Phylogenetic tree constructed by comparative analysis of the *pmoA* gene translated amino acid sequences of strain B3 and other known type II methanotrophs. Bootstrap values exceeding 50% are presented. The *PmoA* fragments of type I methanotrophs are used as an external group. Scale bar indicates 10% differences.

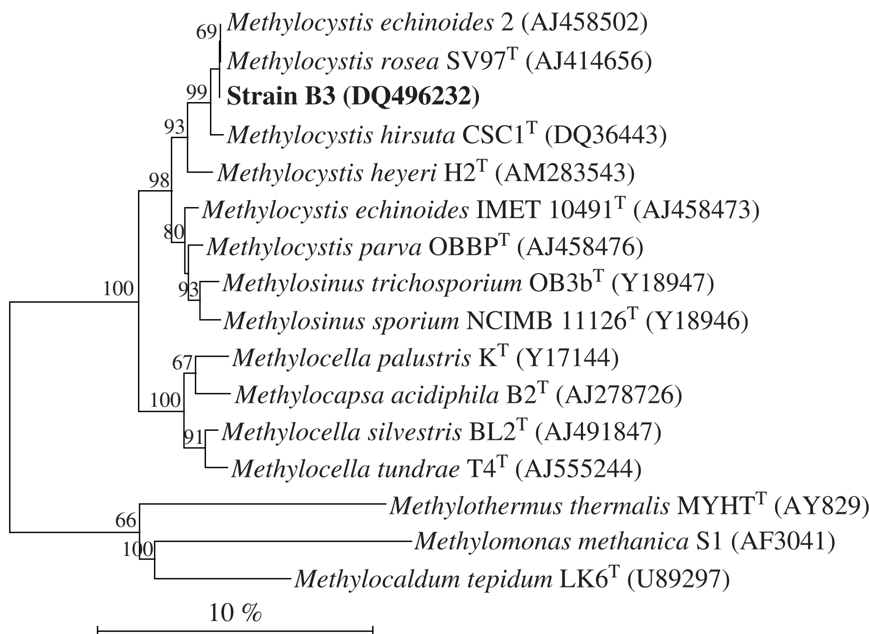

Fig. 2. Phylogenetic tree constructed by comparative analysis of 16S rRNA gene sequences of strain B3 and other known type II methanotrophs. Bootstrap values exceeding 50% are presented. The *PmoA* fragments of type I methanotrophs are used as an external group. Scale bar indicates 10% differences.

ecological niche; these interactions require further investigation. The conditions for isolation of target methanotrophs should possibly consider, apart from their physiological characteristics, the physicochemical parameters of the biotope. A combination of cultural and molecular biological approaches is certainly most efficient for adequate assessment of the real diversity of methanotrophs in extreme biosystems.

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