ISSN 0026-2617, Microbiology, 2011, Vol. 80, No. 5, pp. 713–719. © Pleiades Publishing, Ltd., 2011.
Original Russian Text © N.V. Doronina, E.N. Kaparullina, Yu.A. Trotsenko, 2011, published in Mikrobiologiya, 2011, Vol. 80, No. 5, pp. 700–706.

EXPERIMENTAL ARTICLES

Methylovorus menthalis, a Novel Species of Aerobic Obligate Methylobacteria Associated with Plants

N. V. Doronina, E. N. Kaparullina, and Yu. A. Trotsenko[1]

Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia

Received November 3, 2010

Abstract—A bacterial strain (MM) utilizing methanol as the only carbon and energy source was isolated from corn mint rhizoplane. The cells of the strain were gram-negative colorless motile rods. Spores and prosthecae were not formed, reproduced by binary fission, and did not require vitamins and growth factors. The organism was strictly aerobic, urease-, oxidase-, and catalase-positive. Used the KDPG variant of the ribulose monophosphate pathway. Possessed NAD^+ dependent 6-phosphogluconate dehydrogenase activity and enzymes of the glutamate cycle. The activities of α-ketoglutarate dehydrogenase and of the glyoxylate bypass enzymes (isocitrate lyase and malate synthase) were absent. Palmitic ($C_{16:0}$) and palmitoleic ($C_{16:1}$) acids were predominant in the cell fatty-acid composition. The dominant phospholipids were phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylcholine. The dominant ubiquinone was Q_8. The strain formed indole from tryptophan. The DNA G + C content was 54.5 mol % (T_m). According to the data of the 16S rRNA gene sequencing, strain MM showed high similarity (98–99%) to *Methylovorus glucosotrophus* VKM B-1745^T and *Methylovorus mays* VKM B-2221^T, but the level of DNA–DNA homology with these cultures was only 40 and 58%, respectively. The strain was classified as a new species, *Methylovorus menthalis* sp. nov. (VKM B-2663^T).

Keywords: *Methylovorus menthalis,* aerobic obligate methylobacteria, ribulose monophosphate pathway, rhizoplane.

DOI: 10.1134/S0026261711050043

The ensemble of the root system and soil is a complex ecological niche inhabited by microorganisms, which may be useful, harmful, or neutral to plants. Root cells actively secrete various substances, thereby providing nutrient substrates for the microorganisms forming stable associations with the root, both inside the root tissues and at the root surface (rhizoplane), as well as in the soil immediately around the roots (rhizosphere). Methanol, formaldehyde, formate, methylated amines, and other C_1 compounds are natural products of plant metabolism [1, 2]. Aerobic methylobacteria actively use these C_1 compounds as growth substrates. It has been shown that plant phyllosphere is colonized by aerobic methylotrophic bacteria of different taxonomic groups, which are phytosymbionts synthesizing phytohormones (auxins and cytokinins) and vitamin B_{12} [1, 3].

Rhizospheric and rhizoplane methylotrophs, in contrast to the phyllospheric ones, have been characterized to a much lesser extent. The restricted facultative methylotroph with the ribulose monophosphate metabolic pathway *Methylophius rhizosphaerae* was isolated from rice rhizosphere [4]. The nitrogen-fixing facultative "serine" methylotroph *Crotalaria podocarpa* carrying the nodulation genes was isolated from the root nodules of an African legume *Methylobacterium nodulans* [5]. In general, however, the methylobacteria of plant rhizosphere and rhizoplane are still poorly studied.

The goal of the present work was taxonomic and physiological–biochemical characterization of the new strain of aerobic methylobacteria isolated from corn mint rhizoplane.

MATERIALS AND METHODS

Object of research and cultivation conditions. The root of corn mint (*Mentha arvensis* L.) was dug out of the soil in the vicinity of Pushchino, Moscow oblast, in September 2008. The root was washed three times with sterile distilled water and placed into an Erlenmeyer flask (750 ml) with 200 ml of K medium and 0.5% (vol/vol) methanol. The K medium contained the following (g/l): KH_2PO_4 – 2.0; $(NH_4)_2SO_4$, 2.0; NaCl, 0.5; $MgSO_4 \cdot 7H_2O$ – 0.1; $FeSO_4 \cdot 7H_2O$, 0.002; pH 7.4. After three transfers for 2 days in a shaker (180 rpm) at

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

29°C, the suspension of the methylobacterial enrichment culture was plated to obtaining single colonies (exhaustive inoculation) onto agarized K medium (Difco, United States, 2%) with methanol. Isolated methylobacterial colonies were reinoculated on agar slants, transferred into liquid medium, and then again on solid medium for exhaustive inoculation. Reisolated methylobacterial colonies were reinoculated on slant agar. The purity of the isolated culture was controlled by light and electron microscopy, as well as by the uniformity of colonies on the agarized medium with methanol.

Investigation of cultural, physiological, and biochemical properties of the isolate. The strain was grown on the agarized K medium for describing the colonies and studying cell morphology and motility. The ability of the isolate to reduce nitrates was analyzed in the liquid K medium with ammonium nitrogen replaced by KNO_3 (1 g/l) after 1, 2, and 3 days of incubation. Formation of indole from tryptophan was detected colorimetrically with the Salkowski reagent [6]. The calibration curve was plotted with the standard solutions of indoleacetic acid. Starch hydrolysis was assessed by the reaction with Lugol's solution after culture growth on the agarized K medium with addition of 0.2% (wt/vol) soluble starch. Oxidase was detected with 1% (wt/vol) tetramethyl-*p*-phenylenediamine dihydrochloride solution. Catalase activity was detected by applying 3% hydrogen peroxide solution to streak culture grown on the agarized medium.

Growth temperature range was determined by cultivation in liquid medium with methanol in hermetically sealed flasks on a shaker (120 rpm) at 7–40°C. Growth of the isolate at different pH values was studied in the K medium within the pH range of 5.5–10.0. The pH values were adjusted by adding 1 M NaOH.

The ability of the isolate to utilize various organic compounds as carbon and energy sources was studied as follows: 0.3% (wt/vol) of the analyzed substance was added to the mineral medium instead of methanol and the medium was inoculated with an aliquot of the stationary-phase culture and incubated for 14 days on a shaker at the optimal temperature. All volatile compounds were introduced in the amount of 0.5% by volume.

The spectrum of utilized substrates and some biochemical properties of the tested strain were identified using the Api tests (Api 20E and Api 20NE; Biomerieux, France) according to the manufacturer's protocol. Growth under methane or $H_2 + CO_2 + O_2$ was analyzed as described previously [7].

The ability of the culture to utilize different nitrogen sources was investigated in the K medium, with $(NH_4)_2SO_4$ replaced by the analyzed substances with equimolar amounts of nitrogen. The vitamin requirement of the isolate was studied in the K medium with addition of thiamine · HCl, 50 μg/l; biotin, 50 μg/l; B_{12}, 20 μg/l; or yeast autolysate, 0.01% by volume. The medium without vitamins was used as a control.

Formic acid production in the culture liquid was analyzed using formate dehydrogenase from the methylotrophic yeast *Candida boidinii* (Boehringer, Germany).

Electron microscopy. Electron microscopy of whole cells and ultrathin sections was performed as described previously [8].

Chemotaxonomic analysis. Cellular fatty acid and phospholipid composition of the isolate were determined as described [9]. Ubiquinones were extracted from dry cells, purified by the Collins method [10], and analyzed in a Finnigan MAT 8430 mass spectrometer (Germany). Enzymological analysis was carried out by methods described previously [11].

DNA isolation and analysis. DNA was isolated and purified according to Marmur [12]. The DNA G + C content was determined by heat denaturing in a Beckman DU-8B spectrophotometer (United States) at a heating rate of 0.5°C/min. *Escherichia coli* K-12 DNA was used as a standard. The level of DNA–DNA homology of the strain MM and the type cultures of the genus *Methylovorus* was determined by method of DNA–DNA reassociation [13]. The 16S rRNA gene was amplified by PCR with the universal primers 27f: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492r: 5'-AAGGAAGGTGATCCAGCTCGT-3' for prokaryotic 16S rDNA [14]. A fragment (550 bp) of the *mxa*F gene encoding the large subunit of the classical pyrroloquinoline quinone (PQQ)-dependent methanol dehydrogenase of gram-negative bacteria was amplified using the primers 1003f and 1561r according to the previously described protocol [15].

Reaction products were separated by electrophoresis in 1% agarose gel. Isolation and purification of the DNA fragments from low-melting agarose were carried out in the columns with the Wizard SV Gel and PCR Clean-Up System kit (Promega, United States) according to the manufacturer's protocol. PCR fragments were sequenced using the BigDye® Terminator v1.1 kit and an ABI PRISM® capillary analyzer (Applied Biosystems, United States).

Phylogenetic analysis. Preliminary phylogenetic screening of the similarity of the nucleotide sequences of the 16S rRNA and *mxaF* genes of strain MM according to the GeneBank (NCBI) database was performed using the BLAST software package [http://ncbi.nlm.nih.gov]. For more exact determination of the phylogenetic position, the sequences of the *16S rRNA* and *mxaF* genes were manually aligned with those of the taxonomically close reference strains using the CLUSTAL W software package

[http://www.genebee.msu.su/clustal] and with the relevant sequences available from the latest version of the NCBI Database Project. A rooted phylogenetic tree was constructed by the neighbor-joining method (NEIGHBOR) implemented in the TREECON software package [16]. Evolutionary distances were calculated as number of substitutions per 100 nucleotides. Statistical reliability of branching was estimated by bootstrap analysis of 1000 alternative trees using the appropriate function of the TREECON software package.

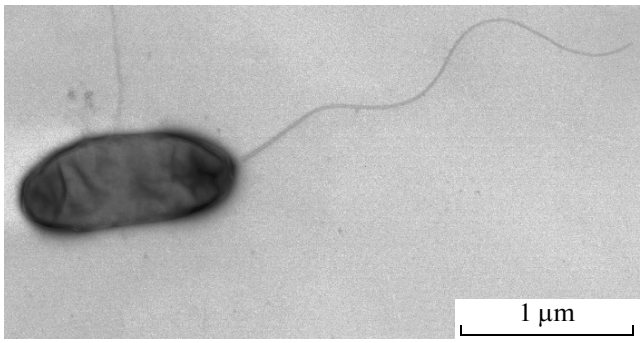

Fig. 1. Morphology of strain MM (negative contrasting).

RESULTS

Morphology. The cells of strain MM were rod-shaped (0.5–0.6 × 1.2–1.3 μm) (Fig. 1) with the cell wall of a gram-negative type. The cells were motile, with monotrichous flagellation, not forming capsules and spores, reproducing by binary fission. On agarized K medium with methanol, they formed pinpoint (up to 0.5 mm in diameter), transparent, colorless, rounded, convex-profiled, smooth-edged colonies of uniform structure with a smooth and glistening surface.

Cultural, physiological, and biochemical properties. In liquid K medium with methanol, the strain under study grew without cell aggregation and did not form pigments. It was strictly aerobic and did not need vitamins. The organism did not grow on methane, methylamine, dimethylamine, trimethylamine, succinate, fumarate, pyruvate, α-ketoglutarate, sucrose, glucose, fructose, xylose, lactose, galactose, glucuronic acid, alanine, glutamate, serine, mannitol, acetamide, acetoin, formamide, dimethyl sulfoxide, or dichloromethane, as well as in $H_2 + CO_2 + O_2$ atmosphere and on malt agar.

Cellulose, gelatin, starch, and casein were not hydrolyzed. The organism was oxidase-, catalase-, and urease-positive; it was able to hydrolyze esculin. Sugars were not fermented. The methyl red, Voges–Proskauer and lipase tests were negative; the β-galactosidase test was positive. Ammonium salts, nitrates, and some amino acids were used as nitrogen sources. Indole derivatives were formed on the medium with nitrates as nitrogen sources, 0.5% methanol, and 1% tryptophan (~10 μg/ml of culture liquid at OD_{600} of the culture 1.4). The temperature and pH growth ranges were 10–37°C and pH 6.0–10.0. Growth temperature optimum was 24–26°C; pH optimum was 8.5–9.0. The culture acidified the medium by producing formic acid (4–7 mM) from methanol.

The main quinone of the MM strain was ubiquinone Q_8. Phosphatidylethanolamine, phosphatidylglycerol and phosphatidylocholine were predominant in the phospholipid composition of the cells. Palmitic ($C_{16:0}$, 48%), palmitoleic ($C_{16:1}$, 35%) and octadecenoic ($C_{18:1}$, 2%) acids were predominant in the fatty acid composition.

The results of enzymological analysis of cells grown on methanol are presented in Table 1. The activities of methanol, formaldehyde (NAD^+- GSH-dependent) and formate (NAD^+) dehydrogenases, hexulosephosphate synthase (the key enzyme of the ribulose monophosphate RuMP pathway), glucose-6-phosphate dehydrogenases (with NAD^+ and $NADP^+$) and 6-phosphogluconate dehydrogenase (NAD^+), 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase, isocitrate dehydrogenase (NAD^+ and $NADP^+$), and the enzymes of the glutamate cycle (glutamate synthase and glutamine synthetase) were revealed. Specific enzymes of the serine pathway (hydroxypyruvate reductase, serine–glyoxylate aminotransferase) and of the Calvin cycle (ribulose-1,5-bisphosphate carboxylase), as well as $NADP^+$-dependent 6-phosphogluconate dehydrogenase, α-ketoglutarate dehydrogenase, glyoxylate bypass enzymes (isocitrate lyase and malate synthase), and glutamate dehydrogenase, were absent. Consequently, the strain MM uses the KDPG variant of the RuMP pathway. Ammonium is assimilated through the system of glutamate cycle enzymes (glutamate synthase, glutamine synthetase).

Genotypic characteristics. Comparative analysis of the 16S rRNA gene nucleotide sequences showed affiliation of the strain MM with the *Betaproteobacteria* and its 98.0–99.0% similarity to the known species of the genus *Methylovorus* (Fig. 2). According to the data of DNA heat denaturation, the G + C content in the tested strain was 54.5 mol%. The level of DNA–DNA homology of the strain with the type representatives of the genus *Methylovorus* – *M. mays* C^T and *M. glucosotrophus* $6B1^T$) was 40 and 58%, respectively.

According to the data of the sequencing of a fragment of the methanol dehydrogenase gene *mxaF* encoding the large subunit of the enzyme, the strain MM showed maximum similarity to representatives of the genera *Methylophilus* and *Methylobacillus* (Fig. 3).

Table 1. Activities of the enzymes of primary and secondary metabolism in the cell extracts of stain MM grown on methanol

Enzyme	Cofactor	Activity, nmol min^{-1} mg^{-1} protein
Methanol dehydrogenase	PMS*	135
Formaldehyde dehydrogenase	PMS	3
	NAD^+	0
	NAD^+, GSH**	38
Formate dehydrogenase	PMS	5
	NAD^+	16
3-Hexulosephosphate synthase		192
Glucose-6-phosphate dehydrogenase	NAD^+	421
	$NADP^+$	1378
6-Phosphogluconate dehydrogenase	NAD^+	230
	$NADP^+$	0
Fructose-1,6-bisphosphate aldolase		0
2-Keto-3-deoxy-6-phosphogluconate aldolase		196
Hydroxypyruvate reductase	NAD(P)H	0
Serine–glyoxylate aminotransferase	NAD(P)H	0
Ribulose-1,5-bisphosphate carboxylase		0
Isocitrate dehydrogenase	NAD^+	58
	$NADP^+$	69
α-Ketoglutarate dehydrogenase	NAD^+	0
Citrate synthase		77
Malate synthase		0
Isocitrate lyase		0
Glutamate dehydrogenase	NAD(P)H	0
Glutamate synthase	NADP	39
	NADPH	19
Glutamine synthetase	ATP, Mn^{2+}	1077

Notes: * PMC is abbreviation for phenazine methosulfate,
** GSM, for reduced glutathione.

DISCUSSION

The strain MM isolated from the rhizoplane of corn mint is an obligate methylotroph utilizing methanol as a carbon and energy source via the KDPG variant of the RuMP cycle. It lacks a number of the central metabolism enzymes, which accounts for its obligate dependence on methanol. Based on the study of its pheno- and genotypic characteristics, the strain MM was affiliated with the genus *Methylovorus* (Table 2). This genus is represented by two species: the restricted facultative methylotroph *M. glucosotrophus* and the obligate methylotroph *M. mays* [17].

The strain MM differs from these species in the higher pH optimum and absence of growth at 40°C. At the same time, it differs from *M. glucosotrophus* VKM B-1745^T in its inability to utilize glucose and hydrolyze starch and in the level of DNA–DNA homology (40%). The level of DNA–DNA homology between the strain MM and the closest species *M. mays* is only 58%. Thus, comparison of the novel strain with the type species of the genus *Methylovorus* makes it possible to propose a new species name for it: *Methylovorus menthalis* sp. nov. MM^T.

The strain MM is most probably not a casual inhabitant of mint rhizoplane. Being obligately dependent on methanol produced by the plant, this methylotroph, in turn, seems to supply the plant with auxins (indole derivatives).

Moreover, this methylotroph produces and secretes formic acid, which may dissolve soil mineral phosphates, making them available for the plant.

DESCRIPTION OF *Methylovorus menthalis* sp. nov.

Methylovorus menthalis sp. nov. (*men. tha' lis*—L. fem. adj.—*menthalis*—a neo-Latin adjective derived from the Latin *mentha*, mint) has been designated by the species name of the host plant *Mentha arvensis* L. (corn mint). The cells are gram-negative, motile, colorless, asporogenous rods (0.5–0.6 × 1.2–

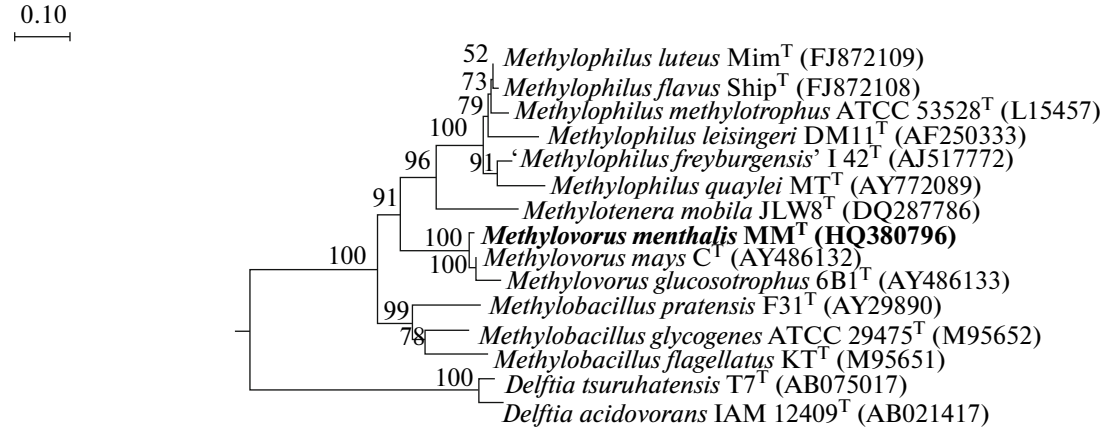

Fig. 2. Phylogenetic position of the strain MM based on comparison of the 16S rDNA nucleotide sequences. The scale corresponds to 10 nucleotide replacements per every 100 nucleotides (evolutionary distance). The root was determined by inclusion of the sequence of *Escherichia coli* 0157:H7 (AY513502) as an outgroup. The numerals show the statistical reliability of the branching order determined by bootstrap analysis of 100 alternative trees.

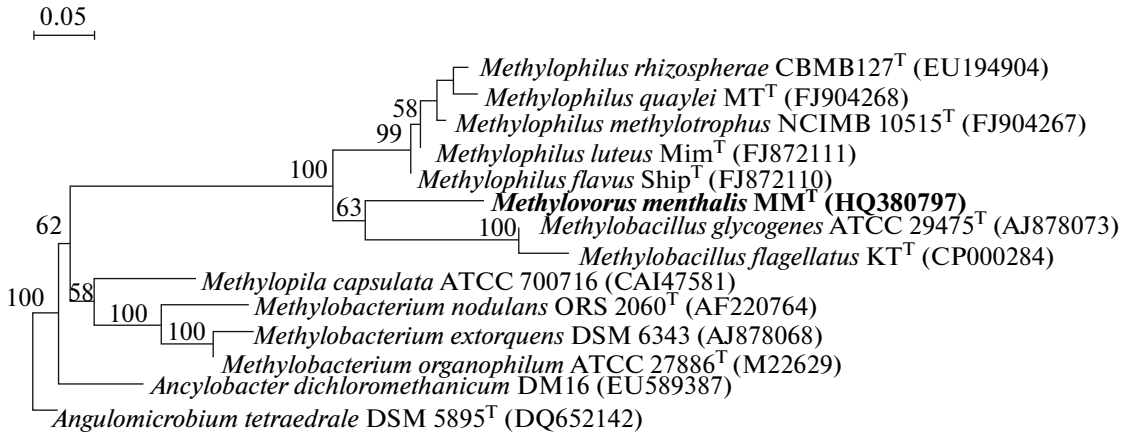

Fig. 3. Phylogenetic position of the strain MM based on comparison of the amino acid sequences of the MxaF protein. The scale corresponds to 5 amino acid substitutions per every 100 amino acids (evolutionary distance). The root was determined by inclusion of the sequence of *Angulomicrobium tetraedrale* as an outgroup. The numerals show the statistical reliability of the branching order determined by bootstrap analysis of 100 alternative trees.

1.3 μm) that are monotrichous, reproducing via binary fission. After 3 days of incubation on agarized mineral medium with methanol at 29°C, the colonies are 0.5 mm in diameter, transparent, colorless, rounded, convex profileLd, smooth edged, with a smooth and glistening surface, of uniform structure. Grows at 10–37°C and pH 6.0–10.0. Growth temperature optimum is 24–26°C; pH optimum is 8.5–9.0. Does not need vitamins or any other growth factors. Does not hydrolyze cellulose, gelatin, casein, or starch; does not produce acetoin, hydrogen sulfide and ammonia. Oxidase-, catalase-, and urease-positive. Strictly aerobic. Reduces nitrates to nitrites. Utilizes only methanol as a carbon and energy source. Nitrogen sources are ammonium salts, nitrates, and some amino acids. Produces indole derivatives on the medium with nitrates as nitrogen sources, 0.5% CH_3OH and 1% tryptophan. Growth is inhibited by 3% NaCl. Uses the KDPG variant of the RuMP pathway. The Krebs cycle is open at the level of α-ketoglutarate dehydrogenase; glyoxylate bypass enzymes (isocitrate lyase and malate synthase) are absent. Assimilates NH_4^+ by the glutamate cycle. Has no activity of $NADP^+$ specific 6-phosphogluconate dehydrogenase. Palmitic ($C_{16:0}$) and palmitoleic ($C_{16:1}$) acids are predominant in the fatty acid composition of the cells. The dominant ubiquinone is Q_8. The dominant phospholipids are

Table 2. Characteristics of the type strains of the genus *Methylovorus*

Character	*M. menthalis* VKM B-2663^T	*M. mays* VKM B-2221^T	*M. glucosotrophus* VKM B-1745^T
Flagella	1	1	1
Methylotrophy type	Obligate	Obligate	Restricted facultative
Growth substrates:			
Methanol	+	+	+
Methylamine	–	–	–
Dimethylamine	–	–	–
Trimethylamine	–	–	–
Glucose	–	–	+
Fructose	–	–	-
Ammonium assimilation	Glutamate cycle (glutamate synthase/glutamate synthetase)		
6-phosphogluconate Dehydrogenase ($NADP^+$)	–	–	–
Optimal growth temperature (°C)	24–26	35–40	35–37
pH optimum	8.5–9.0	7.0–7.5	7.2
G + C content (T_m), mol%	54.5	57.2	55.8
Source of isolation	*Mentha arvensis* L.	*Zea mays* L.	Wastewater

phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylcholine. The DNA G + C content is 54.5% (T_m).

The type strain *M. menthalis* MM^T was isolated from the rhizoplane of corn mint (Pushchino, Moscow oblast, Russia) and deposited at the All-Russian Collection of Microorganisms under the number VKM B-2663^T. The *16S rRNA* and *mxaF* gene sequences of the strain were deposited in the GenBank under accession numbers HQ380796 and HQ380797, respectively.

ACKNOWLEDGMENTS

The work was supported by Federal Contract no. 14.740.11.0111 and the Russian Foundation for Basic Research, project no. 10-04-00808.

REFERENCES

1. Trotsenko, Yu.A., Ivanova, E.G., and Doronina, N.V., Aerobic Methylotrophic Bacteria as Phytosymbionts, *Mikrobiologiya,* 2001, vol. 70, no. 6, pp. 725–736 [*Microbiology* (Engl. Transl.), vol. 70, no. 6, pp. 623–632].
2. Hanson, A.D. and Roje, S., One Carbon Metabolism in Higher Plants, *Ann. Rev. Plant Physiol. and Plant Mol. Biol.*, 2001, vol. 52, pp. 119–137.
3. Trotsenko, Yu.A., Doronina, N.V., and Torgonskaya, M.L., *Aerobnye metilobakterii* (Aerobic Methylobacteria), Gal'chenko, V.F., Ed., Pushchino: ONTI PNTs RAN, 2010, pp. 145–181.
4. Madhaiyan, M., Poonguzhali, S., Kwon, S.-W., and Sa, T.-M., *Methylophilus rhizosphaerae* sp. nov., a Restricted Facultative Methylotroph Isolated from Rice Rhizosphere Soil, *Int. J. Syst. Evol. Microbiol.,* 2009, vol. 59, pp. 2904–2908.
5. Jourand, P., Giraud, E., Bena, G., Sy, A., Willems, A., Gills, M., Dreyfus, B., and De Lajudie, P., *Methylobacterium nodulans* sp. nov., for a Group of Aerobic, Facultatively Methylotrophic, Legume Root-Nodule-Forming and Nitrogen-Fixing Bacteria, *Int. J. Syst. Evol. Microbiol.*, 2004, vol. 54, pp. 2269–2273.
6. Gordon, S.A. and Weber, R.P., Colorimetric Estimation of Indole-Acetic Acid, *Plant Physiol.*, 1951, vol. 26, pp. 192–195.
7. Doronina, N.V., Li, Ts.D., Ivanova, E.G., and Trotsenko, Yu.A., *Methylophaga murata* sp. nov.: a Haloalkaliphilic Aerobic Methylotroph from Deteriorating Marble, *Mikrobiologiya*, 2005, vol. 74, no. 4, pp. 511–519 [*Microbiology* (Engl. Transl.), vol. 74, no. 4, pp. 440–447].
8. Ivanova, E.G. and Doronina, N.V., Trotsenko, Y.A. *Hansschlegelia plantiphila* gen nov. sp. nov., a New Aerobic Restricted Facultative Methylotroph Associated with Plants, *Syst. Appl. Microbiol.,* 2007, vol. 30, no. 6, pp. 444–452.
9. Doronina, N.V., Darmaeva, T.D., and Trotsenko, Y.A., *Methylophaga alcalica* sp. nov., a Novel Alkaliphilic and Moderately Halophilic, Obligately Methylotrophic Bacterium from the East Mongolian Saline Soda Lake, *Int. J. Syst. Evol. Microbiol.*, 2003, vol. 53, pp. 223–229.
10. Collins, M.D, Analysis of Isoprenoid Quinones, in *Methods in Microbiology*, Gottschalk, G., Ed., New York: Academic, 1985, pp. 329–366.
11. Trotsenko, Y.A., Doronina, N.V., and Govorukhina, N.I., Metabolism of Non-Motile Obligately Methylotrophic Bacteria, *FEMS Microbiol. Letts.*, 1986, vol. 33, pp. 293–297.

12. Marmur, J.A., A Procedure for the Isolation of Deoxyribonucleic Acid from Microorganisms, *J. Mol. Biol.*, 1961, vol. 3, pp. 208–214.
13. Doronina, N.V., Govorukhina, N.I., Lysenko, A.M., and Trotsenko, Yu.A., Analysis of DNA–DNA Homology in Obligately Methylotrophic Bacteria, *Mikrobiologiya*, 1988, vol. 57, no. 4, pp. 629–633.
14. Lane, J.D., 16S/23S rRNA Sequencing, in *Nucleic Acid Techniques in Bacterial Systematics*, Stackebrandt, E. and Goodfellow, M., Eds., Chichewster: Wiley, 1991, pp. 115–175.
15. McDonald, I.R. and Murrell, J.C., The Methanol Dehydrogenase Structural Gene *mxa*F and Its Use as a Functional Gene Probe for Methanotrophs and Methylotrophs, *Appl. Environ. Microbiol.*, 1997, vol. 63, no. 8, pp. 3218–3224.
16. Van de Peer, Y. and De Wachter, R., TREECON for Windows: a Software Package for the Construction and Drawing of Evolutionary Trees for the Microsoft Windows Environment, *Comput. Appl. Biosci.*, 1994, vol. 10, pp. 569–570.
17. Doronina, N.V., Ivanova, E.G., and Trotsenko, Y.A., Phylogenetic Position and Emended Description of the Genus *Methylovorus, Int. J. Syst. Evol. Microbiol*, 2005, vol. 55, no. 2, pp. 903–906.