ISSN 0026-2617, Microbiology, 2007, Vol. 76, No. 1, pp. 75–81. © Pleiades Publishing, Ltd., 2007.
Original Russian Text © Ts.D. Li, N.V. Doronina, E.G. Ivanova, Yu.A. Trotsenko, 2007, published in Mikrobiologiya, 2007, Vol. 76, No. 1, pp. 88–94.

EXPERIMENTAL ARTICLES

Vitamin B_{12}-Independent Strains of *Methylophaga marina* Isolated from Red Sea Algae

Ts. D. Li, N. V. Doronina, E. G. Ivanova, 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 February 16, 2006; in final form, May 4, 2006

Abstract—Two strains (KM3 and KM5) of halophilic methylobacteria isolated from Red Sea algae do not require vitamin B_{12} for growth and can use methanol, methylamine, dimethylamine, trimethylamine, dimethyl sulfide, and fructose as sources of carbon and energy. The cells of these strains are gram-negative motile monotrichous (strain KM3) or peritrichous (strain KM5) rods. The strains are strictly aerobic and require Na^+ ions but not growth factors. They are oxidase- and catalase-positive and reduce nitrates to nitrites. Both strains can grow in a temperature range of 4 to 37°C (with optimal growth at 29–34°C), at pH between 5.5 and 8.5 (with optimal growth at pH 7.5–8.0), and in a range of salt concentrations between 0.5 and 15% NaCl (with optimal growth at 5–9% NaCl). The phospholipids of these strains are dominated by phosphatidylethanolamine and phosphatidylglycerol and also include phosphatidylcholine, phosphatidylserine, and cardiolipin. The dominant fatty acids are $C_{16:1\omega7c}$ and $C_{16:0}$. The major ubiquinone is Q_8. The cells accumulate ectoin, glutamate, and sucrose as intracellular osmoprotectants. The strains implement the 2-keto-3-deoxy-6-phosphogluconate–dependent variant of the ribulose monophosphate pathway. The G+C content of the DNA is 44.4–44.7 mol %. Analysis of the 16S rRNA genes showed that both strains belong to *Gammaproteobacteria* and have a high degree of homology (99.4%) to *Methylophaga marina* ATCC 35842^T. Based on the data of polyphasic taxonomy, isolates KM3 and KM5 are identified as new strains *M. marina* KM3 (VKM B-2386) and *M. marina* KM5 (VKM B-2387). The ability of these strains to produce auxins (indole-3-acetic acid) suggests their metabolic association with marine algae.

DOI: 10.1134/S0026261707010110

Key words: restricted facultative methylotrophs, halophiles, marine algae, *Methylophaga marina*, vitamin B_{12}-independent strains.

Higher plants are generally associated with aerobic methylotrophic bacteria because the plants produce volatile C_1-compounds necessary to the bacteria and the bacteria produce phytohormones (cytokinins and auxins) and other growth factors beneficial to the plants [1]. Investigations have shown that various compartments of plant cells contain C_1-metabolites, such as methanol (up to 1 μmol/g wet wt), formate (0.1–1 μmol/g wet wt), and formaldehyde (0.1–10 μmol/g wet wt), which are involved in the biosynthesis of proteins, nucleic acids, pantothenic acid, and a great number of methylated compounds, as well as in the stabilization of pectin in the plant cell walls. Most of the formaldehyde is irreversibly bound to various cellular nucleophilic compounds (glutathione, arginine, and tetrahydrofolate). Methanol and formate can leave the C_1-metabolism of plant cells and be released into the intercellular space and then into the atmosphere via stomates. Methanol can also be converted to formaldehyde [2]. Estimations have shown that the annual emission of methanol by plants reaches 100 million tons, part of which dissolves in the surface layers of oceanic water. The current content of methanol in the world ocean is 228 million tons [3]. It is known that seaweeds produce C_1-compounds, however their colonization by methylotrophic microflora has not yet been studied. Moderately halophilic obligate methylobacteria and restricted facultative methylobacteria of the genus *Methylophaga* are permanent inhabitants of seawater. The known representatives of this genus are vitamin B_{12} auxotrophs [4, 5]. We have recently isolated two strains of moderately halophilic marine methylobacteria which do not require vitamin B_{12} or other factors for growth.

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

The aim of this work was to carry out physiological, biochemical, and taxonomic characterization of the two recently isolated from Red Sea algae.

MATERIALS AND METHODS

Strains and cultivation conditions. Strains KM3 and KM5 were isolated from the green seaweeds *Ulva lactuca* and *Cystoseira trinodes* collected in January 2004 in the near-shore regions of the Red Sea in Egypt. The sampled seaweeds were placed between two sterile sheets of filter paper in a petri dish for dehydration. Then, the seaweeds were used for inoculation of the growth medium of methylobacteria.

The mineral basal medium for the isolation and cultivation of methylobacteria contained (in g/l) KH_2PO_4, 2.0; $(NH_4)_2SO_4$, 2.0; NaCl, 60.0; $MgSO_4 \cdot 7H_2O$, 0.2; and $FeSO_4 \cdot 7H_2O$, 0.002 (pH 7.5). To obtain methylobacteria in enrichment and pure cultures, the medium was supplemented with 1 vol % methanol [6]. The type strain *Methylophaga marina* ATCC 35842^T served as the reference strain.

The study of the cultural, physiological, and biochemical properties of the isolates. To study the colonial properties, morphology, and motility of the isolates, they were grown on agar (Difco) medium with 1 vol % methanol. To study the ability of the isolates to reduce nitrates and to produce indoles, $(NH_4)_2SO_4$ in the medium was replaced with KNO_3. The formation of indoles from L-tryptophan was examined with the Salkowskii reagent [7]. To study the requirement for Na^+ ions, sodium salts in the medium were replaced with the respective K^+, Mg^{2+}, and Li^+ salts. To study their halotolerance, the isolates were grown in medium supplemented with NaCl at various concentrations (from 0 to 20 wt %). The temperatures suitable for growth were determined using liquid medium with 5% NaCl. The inoculated flasks were incubated in a thermostated Clim-o-Shake shaker (System Kuhner, Switzerland) within a temperature range of 0 to 50°C. The pH dependence of growth was studied within a pH range of 6.0 to 12.0. The pH of the medium was adjusted with 1 M NaOH. The hydrolysis of starch, the presence of oxidase and catalase activity, the ability to utilize methane, and the ability to grow autotrophically in an atmosphere of $H_2/O_2/CO_2$ were studied as described elsewhere [6]. To study the ability of the isolates to utilize organic compounds other than methanol as carbon and energy sources, they (a total of 49 compounds were investigated) were added to a methanol-free medium at a concentration of 0.3 wt % (nonvolatile compounds) or 0.5 vol % (volatile compounds). The inoculated media were incubated at an optimum growth temperature on the shaker for two weeks. To study the ability of the isolates to utilize various nitrogen sources, $(NH_4)_2SO_4$ in the medium was replaced with equivalent amounts of other nitrogen sources. Vitamin requirements were studied by adding (separately and in various combinations) thiamine, biotin, folic acid, pyridoxine, riboflavin, nicotinic acid, Ca pantothenate, *p*-aminobenzoic acid, lipoic acid, nicotinamide, and vitamin B_{12} at a concentration of 20 mg/l each or yeast autolysate at a concentration of 0.05 vol %. The control medium contained no vitamins.

Electron microscopy was carried out as described earlier [5].

Chemotaxonomic analysis. The fatty acid and phospholipid profiles of bacterial cells were determined as described earlier [5]. Ubiquinones were extracted, purified by the Collins method [8], and analyzed using a Finnigan MAT-8430 mass-spectrometer (Germany). Indoles were analyzed by TLC and HPLC. After separation by TLC, auxins were determined with the Finnigan MAT-8430 mass-spectrometer. Auxin samples were injected directly at an evaporation temperature of 160°C.

Intracellular osmoprotectants were analyzed by the method of nuclear magnetic resonance [9, 10] using a high-resolution WP 80 SY NMR spectrometer (Bruker, Switzerland).

Enzymes were assayed by routine methods [11].

DNA isolation and analysis. DNA was isolated and purified according to Marmur [12]. The G+C content of the DNA was determined by the method of thermal denaturation at a heating rate of 0.5°C/min using a Beckman DU-8B spectrophotometer and the DNA of *Escherichia coli* K-12 as the standard.

The 16S rRNA genes were amplified by the PCR method using the universal prokaryotic 16S rDNA primers 27f and 1492r (5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-AAGGAAGGTGATCCAGCTCGT-3', respectively). PCR amplifications were carried out in a Hybaid thermal cycler (United Kingdom) with the initial DNA denaturation step at 95°C for 2 min, followed by 25 cycles of DNA denaturation at 94°C for 40 s, primer annealing at 60°C for 40 s, and primer extension at 72°C for 40 s, with the final extension step at 72°C for 4 min. The reaction mixture (30 μl) contained 1 μl DNA preparation and 5 pmol of the respective primer in 10 mM Tris–HCl buffer containing 68 mM $(NH_4)_2SO_4$, 1 mg/ml bovine serum albumin (BSA), and 2.5 mM $MgCl_2$. The mixture was supplemented with 0.2 μM each of dNTP and 1 U *Taq* DNA polymerase. The reaction products were separated by electrophoresis in 1% agarose gel. The PCR products of the 16S rRNA genes were isolated from the agarose gel and purified using a Wizard SV Gel and PCR Clean-Up System kit (Promega, United States). The PCR fragments were sequenced with an automatic CEQ2000 XL sequenator (Beckman Coulter, United States) using a

CEQ Dye Terminator Cycle Sequencing kit (Beckman Coulter).

Phylogenetic analysis. A preliminary phylogenetic screening of the nucleotide sequences of the 16S rRNA genes of strains KM3 and KM5 over the GenBank (NCBI) database was carried out with the aid of the BLAST program package (http://ncbi.nlm.nih.gov). To specify the phylogenetic position of the strains, the nucleotide sequences of their 16S rDNA were manually aligned with the aid of the CLUSTAL W program (http://www.genebee.msu.su/clustal) using the latest relevant sequences of the closest reference strains available in the NCBI Database Project. The rooted phylogenetic tree was constructed by the neighbor-joining method (NEIGHBOR) with the TREECON algorithm [13]. Evolutionary distances were calculated as the number of substitutions per 100 nucleotides. To assess the reliability of branching points on the tree, the sequence data were subjected to bootstrap analysis with the aid of the TREECON program. The nucleotide sequences of the 16S rDNA of strains KM3 and KM5 have been deposited in GenBank under accession numbers DQ400507 and DQ400508, respectively.

RESULTS

Cell morphology. The cells of strains KM3 and KM5 were found to be motile, gram-negative, nonspore-forming rods, 0.4 × 1.3 μm in size, monotrichously (strain KM3) or peritrichously (strain KM5) flagellated. The cells multiply by binary fission and have no complex intracytoplasmic membrane system.

The physiological, biochemical, and chemotaxonomic properties of strains KM3 and KM5. The strains grow in liquid media without cell aggregation. Pigments are not produced. Under optimal conditions, the growth rate was 0.4 h^{-1}. The strains require oxygen but not vitamins for growth. Colonies produced on the agar medium with methanol after three days of incubation at 29°C are round, smooth, even-edged, convex, homogeneous, nonpigmented, translucent, and reach 1 mm in diameter.

Both strains are able to utilize methanol, methylamine, dimethylamine, trimethylamine, dimethyl sulfide, and fructose as sources of carbon and energy and are unable to utilize formaldehyde, formate, organic acids, amino acids, sugars, and C_2–C_6 alcohols when grown on nutrient and malt extract agars or under the atmospheres of $CH_4 + O_2$ and $H_2 + O_2 + CO_2$. Starch is hydrolyzed. Ammonia and hydrogen sulfide are not produced on test media. Indoles (dominated by indole-3-acetic acid) are produced from L-tryptophane. The concentration of indole-3-acetic acid (IAA) reaches a maximum (10–20 μg/ml culture liquid) at the end of the logarithmic growth phase.

Table 1. The fatty acid profiles (in % of the total) of *M. marina* strains grown on methanol at 29°C

Fatty acids	Strains	
	KM3	KM5
$C_{12:0}$	2.8	2.8
$C_{14:0}$	5.5	3.7
$C_{15:0}$	0.9	0.6
$C_{16:0}$	41.8	38.5
$C_{16:1\omega7c}$	40.0	41.1
$C_{17:0}$	0.1	0
$C_{18:0}$	0.3	0.6
$C_{18:1\omega7}$	3.2	8.8
Cyclic fatty acids		
$C_{17:0}$	3.9	3.0
$C_{19:0}$	0.2	0.3
Hydroxy fatty acids		
3-$OHC_{10:0}$	0.2	0.2
3-$OHC_{14:0}$	0.2	0.2

The strains are oxidase- and catalase-positive. Nitrates are reduced to nitrites. The strains are able to utilize nitrates, glutamate, urea, methylamines, and ammonium ions as nitrogen sources. They can grow in liquid media at temperatures between 4 and 37°C (with optimal growth at 29–34°C) and pH between 5.5 and 8.5 (with optimal growth at 7.5–8.0) and obligately require Na^+ ions for growth, as is evident from the fact that growth was not observed in the media in which

Table 2. Enzyme activities (in nmol/min mg protein) in extracts of the methanol-grown cells of *M. marina* KM3 and KM5

Enzyme	Cofactor	Strains	
		KM3	KM5
Methanol dehydrogenase	PMS	74	139
Formaldehyde dehydrogenase	PMS	69	59
	NAD^+	55	27
Formate dehydrogenase	PMS	57	36
	NAD^+	50	35
Hydroxypyruvate reductase	NAD(P)H	0	0
Serine–glyoxylate aminotransferase	NAD(P)H	0	0
Ribulose-1-5-bisphosphate carboxylase		0	0
3-Hexulose-6-phosphate synthase		170	181
Glucose-6-phosphate dehydrogenase	NAD^+	605	153
	$NADP^+$	665	700
6-Phosphogluconate dehydrogenase	NAD^+	268	27
	$NADP^+$	89	108
2-Keto-3-deoxy-6-phosphogluconate aldolase		87	96
Fructose-1,6-bisphosphate aldolase		0	0
6-Phosphofructokinase	ATP, PPi	0	0
Transaldolase		80	74
Transketolase		120	105
2-Oxoglutarate dehydrogenase	NAD^+	0	0
Isocitrate dehydrogenase	NAD^+	0	0
	$NADP^+$	131	154
Isocitrate lyase		0	0
Malate synthase		0	0
Glutamate dehydrogenase	NADH	79	47
	NAD(P)H	108	108
Glutamine synthetase	ATP, Mn^{2+}	135	112
Glutamate synthase	NADH	19	32
	NAD(P)H	99	150

Note: PMS stands for phenazine methosulfate.

sodium salts were replaced with the respective potassium, magnesium, or lithium salts. The strains are able to grow at NaCl concentrations up to 15% (with optimal growth at 5–9% NaCl), suggesting that they are moderate halophiles. The strains can grow at methanol concentrations up to 7 vol % (with optimal growth at 0.5 vol % methanol). Ubiquinone Q_8 was found to be the major quinone in both strains. Cellular phospholipids are dominated by phosphatidylethanolamine and phosphatidylglycerol and also include phosphatidylcholine, phosphatidylserine, and cardiolipin. The dominant fatty acids are $C_{16:1\omega7c}$ and $C_{16:0}$ (Table 1). Like other methylobacteria from the genus *Methylophaga*, the strains synthesize ectoin (1,4,5,6-tetrahydro-2-methyl-4-pirimidine carboxylic acid), glutamate, and sucrose as intracellular osmoprotectants (the first two osmoprotectants are dominant). The concentration of the osmoprotectants increases with increasing osmolarity of the growth medium. In the medium with 9% NaCl, the content of ectoin in the cells is as high as 20% of dry weight.

The assay of enzymes in the methanol-grown cells of strains KM3 and KM5 (Table 2) showed the presence of methanol dehydrogenase, formaldehyde dehydrogenase, formate dehydrogenase, and 3-hexulose-6-phosphate synthase (the key enzyme of the ribulose monophosphate pathway), as well as glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, transaldolase, transketolase, and 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase. The specific enzymes of the serine pathway (hydroxypyruvate reductase and serine–glyoxylate aminotransferase) and the Calvin cycle (ribulose-1,5-bisphosphate carboxylase) were not detected. Consequently, strains KM3 and KM5 implement the KDPG variant of the ribulose monophosphate (RuMP) pathway. 2-Oxoglutarate dehydrogenase, 6-phosphofructokinase, fructose-1,6-bisphosphate aldolase, isocitrate lyase, and malate synthase were not detected either. Ammonium ions were assimilated through the reductive amination of 2-oxoglutarate and via the glutamate cycle with the involvement of glutamate synthase/glutamine synthetase.

Genotypic characterization. The G+C content of the DNA was found to be 44.4 mol % in strain KM3 and 44.7 mol % in strain KM5. The 16S rRNA gene sequencing showed that strains KM3 and KM5 belong to the class *γ-Proteobacteria* and have a high degree of homology between each other (99.8%) and to the type strain *Methylophaga marina* ATCC 35842^T, suggesting that both strains belong to the genus *Methylophaga*.

DISCUSSION

The aerobic, moderately halophilic, gram-negative methylobacteria under study were isolated from two seaweed species sampled in two different regions of the

Red Sea. They belong to a group of restricted facultative methylotrophs because, in addition to C_1-compounds, they can utilize fructose, implement the RuMP pathway, and have multiple defects in the central metabolic pathways (the TCA cycle and the glyoxylate cycle).

The Red Sea water has a high content of NaCl (up to 8 wt %). The isolated strains are obviously well adapted to this water because show optimal growth at 5–9 wt % NaCl. In this case, they synthesize de novo intracellular osmoprotectants (ectoine, glutamate, and sucrose). The content of these compounds in the methylobacterial cells depends on the NaCl concentration in the medium and is directly proportional to external salinity.

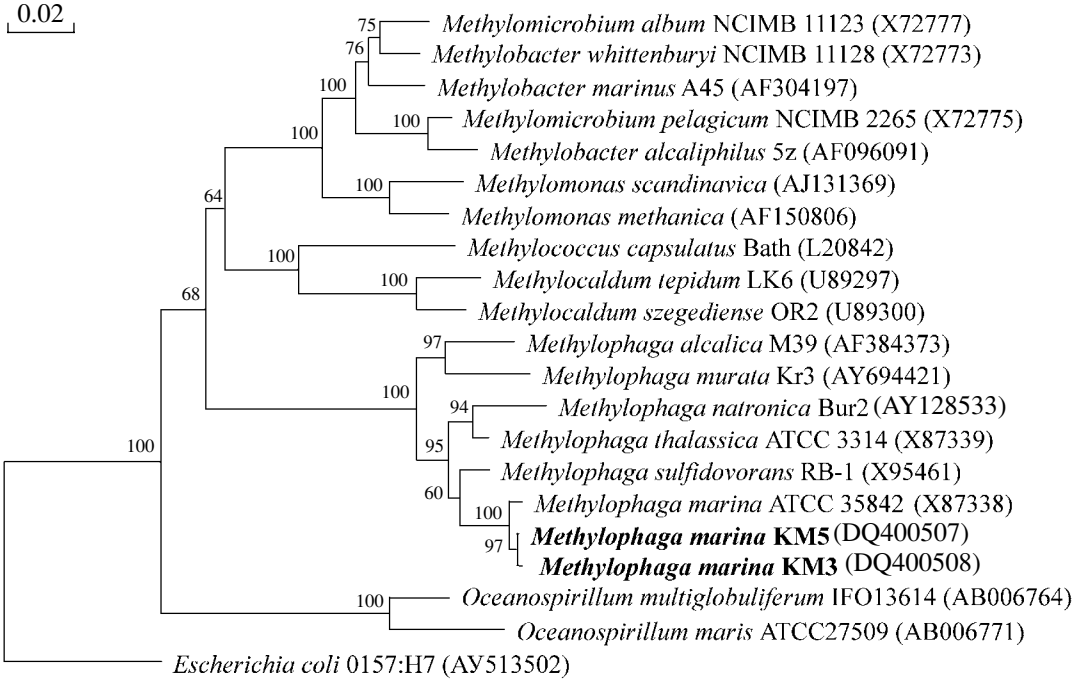
When grown in the medium with methanol as the carbon and energy source and nitrates as the nitrogen source, both strains produce indoles (primarily, indole-3-acetic acid) from L-tryptophan, i.e., the auxin phytohormones. In contrast, when grown in the medium with ammonium salts as the nitrogen source, the strains do not produce indoles, presumably due to the fact that ammonium inhibits the deamination of tryptophan. The ability of the newly isolated and other *Methylophaga marina* strains to synthesize auxins [14] suggest the metabolic association of these methylobacteria with seaweeds.

Methylophaga is the only known genus of moderately halophilic, aerobic, gram-negative methylobacteria implementing the RuMP pathway. The neutrophilic representatives of this genus (*M. marina*, *M. thalassica* [4], and *M. sulfidovorans* [15]) were isolated from seawater; the alkaliphilic species *M. alcalica* [5] and *M. natronica* [6], from soda lakes; and the alkalitolerant species *M. murata*, from deteriorating marble [6].

Bacteria of the genus *Methylophaga*, which belong to *γ-Proteobacteria*, are obligate or restricted facultative methylotrophs. They have no 2-oxoglutarate dehydrogenase, possess ubiquinone Q_8 and fatty acids $C_{16:0}$ and $C_{16:1}$ in their profiles, and show a relatively low G+C content of DNA (43–49 mol %). According to all these characteristics, strains KM3 and KM5 are close to methylobacteria of the genus *Methylophaga.* However, unlike the known *Methylophaga* species, the new isolates do not require vitamin B_{12} for growth. Moreover, vitamin B_{12} does not even stimulate their growth. The comparative characteristics of the new isolates and the type *M. marina* strain are listed in Table 3. The phylogenetic position of *M. marina* KM3 and KM5 among the known *Methylophaga* species and other members of the class γ-*Proteobacteria* is shown in the figure. The new strains *M. marina* KM3 and *M. marina* KM5 isolated from the Red Sea algae are deposited in the All-Russian Collection of Micro-

Table 3. Differentiating characteristics of the new isolates and the type strain of *Methylophaga marina*

Characteristic	Strain KM3	Strain KM5	*Methylophaga marina* ATCC 38842^T
Cell size, μm	0.4–1.3	0.4–1.3	0.2–1.0
Type of methylotrophy	Restricted facultative	Restricted facultative	Restricted facultative
Utilization of:			
fructose	+	+	+
methanol	+	+	+
methylamine	+	+	+
dimethylamine	+	+	+
trimethylamine	+	+	+
dimethyl sulfide	+	+	+
C_1 assimilation pathway	RuMP	RuMP	RuMP
Vitamin requirement	–	–	B_{12}
Reduction of NO_3^- to NO_2^-	+	+	–
Temperature range, °C (optimum)	4–37 (29–34)	4–37 (29–34)	10–40 (30–37)
pH range (optimum)	5.5–8.5 (7.5)	5.5–8.5 (7.5–8.0)	5.0–9.0 (7.0–7.5)
Maximum NaCl concentration (%) for growth (optimum)	15 (5–9)	15 (5–9)	12 (1–4)
Major ubiquinone	Q_8	Q_8	Q_8
Major fatty acids	$C_{16:0}$, $C_{16:1\omega7c}$	$C_{16:0}$, $C_{16:1\omega7c}$	$C_{16:0}$, $C_{16:1}$
G+C content, mol %	44.4	44.7	43.0



The phylogenetic position of *Methylophaga marina* KM3 and KM5 among the γ-*Proteobacteria* methylotrophs. The scale bar corresponds to two nucleotide substitutions per 100 nucleotides. The statistical significance of branching points on the tree was assessed by bootstrap analysis.

organisms as VKM B-2386 and VKM B-2387, respectively.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, grant no. 06-04-49498, and the Ministry of Education and Science of the Russian Federation, grant RNP 2.1.1.2671.

REFERENCES

1. Trotsenko, Yu. A., Ivanova, E.G., and Doronina, N.V., Aerobic Methylotrophic Bacteria as Phytosymbionts, *Mikrobiologiya*, 2001, vol. 70, no. 6, pp. 808–830.
2. Hanson, A.D. and Roje, S., One-Carbon Metabolism in Higher Plants, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 2001, vol. 52, pp. 119–137.
3. Galbally, I.E. and Kirstine, W., The Production of Methanol by Flowering Plants and the Global Cycle of Methanol, *J. Atmos. Chem.*, 2002, vol. 43, pp. 195–229.
4. Janvier, M., Frehel, C., Grimont, F., and Gasser, F., *Methylophaga marina* gen. nov., sp. nov. and *Methylophaga thalassica* sp. nov., Marine Methylotrophs, *Int. J. Syst. Bacteriol.*, 1985, vol. 35, pp. 131–139.
5. Doronina, N.V., Darmaeva, T.D., and Trotsenko, Y.A., *Methylophaga alcalica* sp. nov., a New Alkaliphilic and Moderately Halophilic, Obligately Methylotrophic Bacterium from the East Mongolian Soda Lake, *Int. J. Syst. Evol. Microbiol.*, 2003, vol. 53, pp. 223–229.
6. 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.
7. Gordon, S.A. and Weber, R.P., Colorimetric Estimation of Indole Acetic Acid, *Plant Physiol.*, 1951, vol. 26, pp. 192–195.
8. Collins, M.D., Analysis of Isoprenoid Quinones, *Methods Microbiol.*, 1985, vol. 18, pp. 329–366.
9. Doronina, N.V., Trotsenko, Y.A., and Tourova, T.P., *Methylarcula marina* gen. nov., sp. nov. and *Methylarcula terricola* sp. nov.: Novel Aerobic, Moderately Halophilic, Facultatively Methylotrophic Bacteria from Coastal Saline Environments, *Int. J. Syst. Evol. Microbiol.*, 2000, vol. 50, pp. 1849–1859.
10. Khmelenina, V.N., Kalyuzhnaya, M.G., Sakharovsky, V.G., Suzina, N.E., Trotsenko, Y.A., and Gottschalk, G., Osmoadaptation in Halophilic and Alkaliphilic Methanotrophs, *Arch. Microbiol.*, 1999, vol. 172, pp. 321–329.
11. Trotsenko, Y.A., Doronina, N.V., and Govorukhina, N.I., Metabolism of Nonmotile Obligately Methylotrophic Bacteria, *FEMS Microbiol. Letts.*, 1986, vol. 33, pp. 293–297.
12. Marmur, J., A Procedure for the Isolation of Deoxyribonucleic Acid from Microorganisms, *J. Mol. Biol.*, 1961, vol. 3, pp. 208–214.

13. Van de Peer, Y. and De Watcher, 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. 596–570.

14. Doronina, N.V., Ivanova, E.G., and Trotsenko, Yu.A., New Evidence for the Ability of Methylobacteria and Methanotrophs to Synthesize Auxins, *Mikrobiologiya*, 2001, vol. 70, no. 6, pp. 905–908.

15. Zwart de, J.M.M., Nelisse, P.N., and Kuenen, J.G., Isolation and Characterization of *Methylophaga sulfidovorans* sp. nov.: An Obligately Methylotrophic, Aerobic, Dimethylsulfide-oxidizing Bacterium from a Microbial Mat, *FEMS Microbiol. Ecol.*, 1996, vol. 20, pp. 261–270.

16. Doronina, N.V., Darmaeva, T.D., and Trotsenko, Y.A., *Methylophaga natronica* sp. nov., a New Alkaliphilic and Moderately Halophilic, Restricted Facultatively Methylotrophic Bacterium from Soda Lake of the Southern Transbaikal Region, *Syst. Appl. Microbiol.,* 2003, vol. 26, pp. 382–389.