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EXPERIMENTAL ARTICLES

Novel Aerobic Methylotrophic Isolates from the Soda Lakes of the Southern Transbaikal Region

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Abstract—Twenty-one bacterial associations isolated from the soda lakes of the southern Transbaikal region were found to be able to actively grow at pH 9–10 on methanol as the source of carbon and energy. Two alkalitolerant facultatively methylotrophic strains, Bur 3 and Bur 5, were obtained in pure cultures. Both strains represent gram-negative, nonmotile, bean-shaped, encapsulated cells that reproduce by binary fission. The strains are able to grow at temperatures ranging from 6 to 42°C, with an optimum growth temperature of 25–29°C (strain Bur 3) and 35–37°C (strain Bur 5) and at pH between 6.5 and 9.5, with an optimum pH value of 8.0–8.5. At pH 9.0, strain Bur 3 exhibits an increased content of phosphatidylglycerol and a decreased content of phosphatidylethanolamine. Strains Bur 3 and Bur 5 are similar in the G+C content of their DNAs (66.2 and 65.5 mol %, respectively) and in the type of the dominant ubiquinone (Q_{10}). Unlike Bur 5, strain Bur 3 is able to grow autotrophically in an atmosphere of $CO_2 + O_2 + H_2$. The strains oxidize, by the respective dehydrogenases, methanol to CO_2, which is assimilated by the ribulose bisphosphate pathway. Ammonium ions are assimilated in the glutamate cycle and by the reductive amination of α-ketoglutarate. The strains are highly homologous to each other (92%) and are much less homologous (at a level of 28–35%) to representatives of the genus *Ancylobacter, A. aquaticus* ATCC 25396^T and *A. vacuolatum* DSM 1277. Based on the results obtained, both strains are assigned to a new species, *Ancylobacter natronum* sp. nov.

Key words: soda lakes, aerobic alkalitolerant methylobacteria, *Ancylobacter natronum*.

The investigation of the functional and phylogenetic diversity of alkaliphilic prokaryotes in soda lakes was initiated, to a certain degree, by the hypothesis that alkaliphilic microbial communities represent relics of the Early Proterozoic era, when epicontinental bodies of water might serve as sources of diverse terrestrial microbiota [1].

To answer the question of whether or not an alkaliphilic microbial community can serve as a source of diverse terrestrial microflora, it is necessary (1) to study the structure and representability of this community, i.e., the ability of the member species of this community to represent all the main branches of the phylogenetic tree of prokaryotes and (2) to investigate the taxonomic, structural, and functional relationships between alkaliphilic and neutrophilic bacteria. The shallow-water soda lakes of the southern Transbaikal region are characterized by a wide range of seasonal atmospheric temperatures (from –40 to +40°C); this leads to vertical gradients in the salinity of lake waters and their complete freezing in winter. Therefore, these bodies of water can be used to study the phylogeny, taxonomic diversity, and the survival mechanisms of prokaryotes under the conditions of the action of high pH values (from 8.5 to 10.0) and other stress factors [1].

The microbial communities of soda lakes have been found to represent multicomponent ecosystems with steady trophic relationships and an almost closed cycle of organic matter [2]. Recent studies of the microflora of the soda lakes of the southern Transbaikal region have revealed a wide range of prokaryotes: cyanobacteria, purple sulfur and nonsulfur bacteria, proteolytics, saccharolytics, cellulolytics, sulfate reducers, methanogens, and methanotrophs [1–7]. However, the presence of aerobic methylotrophic bacteria capable of utilizing oxidized and substituted methane derivatives has not yet been reported.

The aim of the present work was to isolate and characterize aerobic methylobacteria from the soda lakes of the southern Transbaikal region.

MATERIALS AND METHODS

Samples of bottom sediments from the soda lakes of the southern Transbaikal region (Barun Torei, Ostozhe, Ilim-Torum, Malyi Kasytui, Dabsu-Nur, Khilgantyn, Tsaidam, Selenduma, Sul'fatnoe, Bezymyannoe, Suduntuiskii Torom, Bulamai Nur, Maloe Guzhirnoe, Khuzhirta, Srednyaya Gorbunka, Malaya Gorbunka, Khilganta, Verkhnee Beloe, Nizhnee Beloe, and Kha-

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dyn) were a generous gift from B.B. Namsaraev, Institute of General and Experimental Biology of the Siberian Division of the Russian Academy of Sciences in Ulan-Ude, and D.Yu. Sorokin, Institute of Microbiology of the Russian Academy of Sciences in Moscow. The hydrochemical characteristics of these lakes were described earlier [1].

To obtain aerobic methylobacteria in enrichment cultures, we used mineral M medium of the following composition (g/l): KNO_3, 1.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 6H_2O$, 0.02; $NaHCO_3$, 8.4; Na_2CO_3, 3.0; and 1 ml/l of a trace element solution containing (g/l) EDTA, 5.0; $FeSO_4 \cdot 7H_2O$, 2.0; $ZnSO_4 \cdot 7H_2O$, 0.1; $MnCl_2 \cdot 4H_2O$, 0.03; $CoCl_2 \cdot 6H_2O$, 0.2; $CuCl_2 \cdot 6H_2O$, 0.1; $NiCl_2 \cdot 6H_2O$, 0.02; $NaMoO_4$, 0.03 (pH 9.5). Phosphates and carbonates were sterilized separately at 1 atm for 30 min and added to the medium just before inoculation. The medium was supplemented with 0.5 vol % methanol as the source of carbon and energy and 0.05 vol % yeast autolysate as the source of vitamins. Cultivation was performed in 750-ml flasks with 100 ml of the medium inoculated with 0.5 vol % of bottom sediment samples and incubated at 29°C on a rotary shaker (140 rpm) for 1 week. Then 10-ml culture aliquots were transferred to fresh media with methanol and incubated in the same way. Aerobic methylobacteria were obtained in pure cultures using M medium supplemented with 1.5% methanol and solidified by adding 1.5% Difco agar. The individual colonies of methylobacteria were transferred to agar slants. Culture purity was tested by visually examining the morphology of colonies grown on agar plates with peptone and methanol or glucose as the sources of nitrogen and carbon. In addition, culture purity was tested by the light or electron microscopy of cultures grown in the liquid medium.

Alternatively, methylotrophic isolates and reference strains were grown in B medium of the following composition (g/l): KH_2PO_4, 1.4; $(NH_4)_2SO_4$, 3.0; $MgSO_4 \cdot 7H_2O$, 0.02; and Na_2HPO_4, 3.0 (pH 7.2–8.0). After sterilization at 1 atm for 30 min, the medium was supplemented, in an amount of 1 ml/100 ml medium, with a trace element solution containing (mg/100 ml) Fe,NH_4 citrate, 300; $CaCl_2 \cdot 2H_2O$, 300; $MnCl_2 \cdot 4H_2O$, 50; $ZnSO_4 \cdot 7H_2O$, 50; and $CuSO_4 \cdot 5H_2O$, 5.

Ancylobacter aquaticus ATCC 25396^T, *Angulomicrobium tetraedrale* Z-2821 [8], and *Ancylobacter vacuolatum* DSM 1277 (formerly *Renobacter vacuolatum*) [9] were used as reference strains. The optimum and marginal growth temperatures of methylobacteria were determined by incubating them at different temperatures on a Clim-o-Shake temperature-controlled shaker (System Kuhner, Switzerland) for 3 days. The ability of the isolates to produce indole was tested using the Sal'kovskii reagent [10]; in this case, the isolates were grown in B medium with 0.1 wt % L-tryptophan and 0.5% methanol to the late logarithmic phase.

DNA was isolated using sodium lauryl sulfate, proteinase K, RNase, and cetyltrimethylammonium bromide. The melting temperature of DNA was determined by recording the first-derivative spectrum of a DNA solution heated at a rate of 0.5°C in a Pye Unicam SP1800 spectrophotometer (the United Kingdom). The G+C content of DNA was calculated by the formula G+C (mol %) = $2.08T_m - 106.4$. DNA–DNA hybridization was performed by the Denhardt method [11] at 57°C for 24 h using a 35% solution of formamide. High-polymeric DNA was immobilized on 0.23-μm-pore-size nitrocellulose filters 100 mm in diameter (Hiyu Kalur, Estonia). DNA was labeled by nick-translation using the necessary reagent kits and deoxy[^{3}H]adenosine 5'-triphosphate purchased from Amersham (the United Kingdom).

The morphological, cultural, physiological, and biochemical properties of the isolates, as well as the composition of their ubiquinones and phospholipids and the activity of some of their enzymes, were determined as described earlier [12]. To quantify phospholipids, bacteria were grown in 100 ml of a medium containing 0.5% methanol and 4 μCi $^{14}CH_3OH$. Cell extracts were subjected to thin-layer chromatography on Kieselgel 60 plates (Merck, Germany). The developed plates were stained with iodine, and the visualized zones of the silica gel adsorbent containing phospholipids were scraped into vials with 5 ml of a scintillation cocktail (4 g of 2,5-diphenyloxazole and 0.05 g of 1,3-bis[5-phenyl-2-oxazolyl]-benzene in 1 l of toluene). The radioactivity of samples was quantified with an SL-30 scintillation spectrometer (Intertechnique, France).

RESULTS

Twenty-one bacterial associations obtained from the bottom sediment samples of the soda lakes of the southern Transbaika region were able to grow at pH 9.0–10.0 in M medium with methanol as the sole source of carbon and energy. We also succeeded in isolating two methylotrophic strains, Bur 3 and Bur 5, in pure cultures from the sediment samples taken from lakes Maloe Guzhirnoe and Srednyaya Gorbunka, respectively.

Strain Bur 3 represents gram-negative, nonmotile, bean-shaped, encapsulated cells 0.4–0.5 × 1.1 μm in size (Fig. 1a), which reproduce by binary fission (Fig. 1b). Three-day-old colonies of this strain grown on nutrient agar are small (below 1 mm in diameter), rounded, colorless, translucent, with a smooth surface, even edge, and viscous consistency. Obligate aerobe. Requires biotin for growth. It utilizes methanol, glucose, arabinose, galactose, maltose, rhamnose, ribose, raffinose, sucrose, betaine, glutamate, dimethylglycine, serine, adonitol, dulcitol, glycerol, inositol, mannitol, sorbitol, ethanol, acetate, acetamide, malate, and glucuronic acid as sources of carbon and energy. Poor growth is observed on formate, 0.02% formaldehyde, xylose, fructose, alanine, arginine, aspartate, dimethylsulfoxide, acetone, and succinate, but not on valine, glycine, tryptophan, methylamine, dimethylamine, trimethylamine, formamide, citrate, isocitrate, and inulin. It is

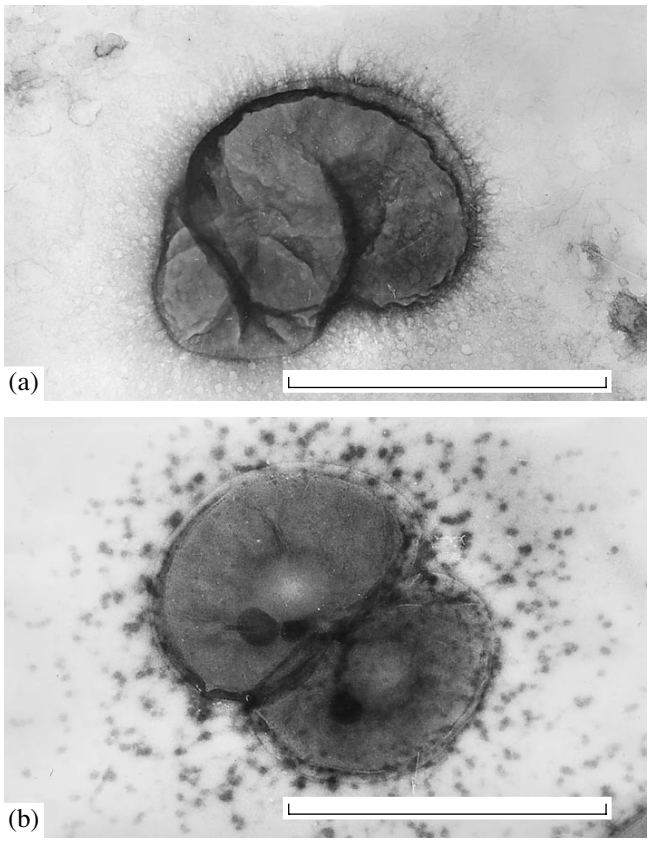


Fig. 1. Morphology of strain Bur 3: (a) an encapsulated bean-shaped cell and (b) formation of the septum between two daughter cells. Bars represent 1 μm.

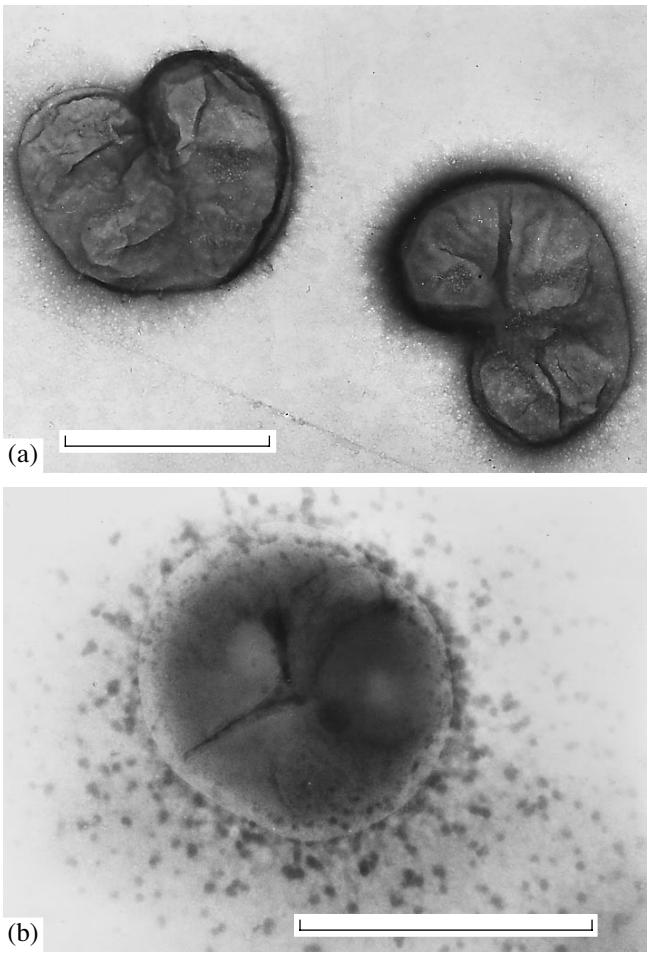


Fig. 2. Morphology of strain Bur 5: (a) encapsulated bean-shaped cells and (b) dividing circular cell. Bars represent 1 μm.

able to grow autotrophically in an atmosphere of CO_2 + H_2 + O_2 but not in an atmosphere of CH_4 + O_2. Glutamate, $(NH_4)_2SO_4$, KNO_3, and aspartate are utilized as nitrogen sources.

The optimum concentration of NaCl for the growth of strain Bur 3 is 0.75%. Higher salt concentrations inhibit its growth partially, while 2% NaCl inhibits its growth completely.

Strain Bur 3 is susceptible to a wide range of antibiotics, such as ampicillin, gentamicin, kanamycin, lincomycin, nalidixic acid, neomycin, novobiocin, streptomycin, and erythromycin, taken in an amount of 30 μg/disk.

Starch and gelatin are hydrolyzed. Nitrates are reduced to nitrites. Indole, ammonia, and hydrogen sulfide are produced. The Voges–Proskauer and methyl red tests are negative. Catalase- and oxidase-positive. Urease is absent. When grown aerobically (but not anaerobically) in a medium with glucose, strain Bur 3 acidifies the medium.

Strain Bur 3 is able to grow at temperatures ranging from 6 to 37°C, with an optimum growth temperature of 25–29°C, and at a pH level between 6.5 and 9.5, with an optimum pH value of 8.0–8.5. The major ubiquinone is Q_{10}; ubiquinone Q_9 is present in a minor amount. Phospholipids are dominated by phosphatidylethanolamine and phosphatidylcholine; phosphatidylglycerol, phosphatidylserine, and cardiolipin are present in minor amounts. Phosphatidic acid was not detected. The relative content of phospholipids in cells depends on the pH of the medium: at high pH values, cells contain more phosphatidylglycerol and less phosphatidylethanolamine as compared with low pH values (Table 1). The G+C content of DNA is 66.2 mol %.

Strain Bur 5 represents gram-negative, nonmotile, bean-shaped, encapsulated cells 1.4 × 0.7 μm in size (Fig. 2a), which reproduce by binary fission with the formation of circular structures (Fig. 2b). Three-day-old colonies of this strain grown on nutrient agar are small (below 1 mm in diameter), rounded, colorless, translucent, with a smooth surface, even edge, and viscous consistency. Obligate aerobe. Requires a mixture of vitamins (thiamine, biotin, and pantothenic acid) or yeast autolysate for growth. Utilizes methanol, glucose, arabinose, galactose, maltose, rhamnose, ribose, raffinose, sucrose, betaine, glutamate, dimethylglycine, serine, adonitol, dulcitol, glycerol, mannitol, sorbitol,

ethanol, acetate, acetamide, malate, and glucuronic acid as sources of carbon and energy. Poor growth is observed on formate, 0.02% formaldehyde, xylose, fructose, succinate, inulin, malate, and acetate but not on valine, glycine, tryptophan, methylamine, dimethylamine, trimethylamine, formamide, dimethylsulfoxide, acetone, citrate, isocitrate, inulin, inositol, and fumarate. Is unable to grow in atmospheres of $CO_2 + H_2 + O_2$ and $CH_4 + O_2$. $(NH_4)_2SO_4$, KNO_3, glutamate, and alanine are utilized as the sources of nitrogen.

The optimum concentration of NaCl for the growth of strain Bur 5 is 0.3–0.5%. NaCl at a concentration of 1% completely inhibits the grown of this strain.

Starch, but not gelatin, is hydrolyzed. Nitrates are reduced to nitrites. Indole and ammonia, but not acetylmethylcarbinol, are produced. Catalase- and oxidase-positive. Urease is absent. When grown aerobically (but not anaerobically) in a medium with glucose, strain Bur 5 acidifies the medium.

The strain is able to grow at temperatures ranging from 4 to 42°C, with an optimum growth temperature of 35–37°C, and at pH between 6.5 and 9.5, with an optimum pH value of 8.0–8.5. The major ubiquinone is Q_{10}; ubiquinone Q_9 is present in a minor amount. Phospholipids are dominated by phosphatidylethanolamine and phosphatidylglycerol; phosphatidylcholine, phosphatidylserine, and cardiolipin are present in minor amounts. The G+C content of DNA is 65.5 mol %.

Strain Bur 5 is susceptible to ampicillin, gentamicin, kanamycin, lincomycin, and streptomycin taken in an amount of 30 μg/disk and is resistant to nalidixic acid, neomycin, novobiocin, and erythromycin.

To elucidate the primary pathways for the oxidation of C_1-compounds, we measured the activity of methanol, formaldehyde, and formate dehydrogenases. Carbon assimilation was analyzed by measuring the activity of the key enzymes of the serine, ribulose monophosphate (RuMP), and ribulose bisphosphate (RuBP) pathways. Central metabolism was studied by evaluating the activity of the enzymes of carbon metabolism and the Krebs cycle. The primary assimilation of ammonium was investigated by assaying glutamate dehydrogenase, glutamate synthase, and glutamate synthetase. The mean values of enzymatic activities in the soluble fraction of methanol-grown cells are presented in Table 2.

As is evident from the data presented in this table, both methylotrophic strains derive energy from the direct oxidation of methanol to CO_2 in a sequence of reactions catalyzed by methanol dehydrogenase, formaldehyde dehydrogenase dependent on NAD^+ and reduced glutathione (GSH), and two formate dehydrogenases dependent on NAD^+ and phenazine methosulfate (PMS).

Cells also exhibited the activities of phosphoribulokinase and ribulose bisphosphate carboxylase, the key enzymes of the RuBP pathway. In spite of the presence of oxypyruvate reductase in a minor amount, the absence of serine–glyoxylate aminotransferase, malate lyase, and hexulose-6-phosphate synthase indicates that the serine and RuMP pathways do not function. Therefore, both strains utilize methanol by oxidizing it to CO_2, which is then assimilated in the RuBP pathway. We also detected fructose-bisphosphate aldolase but failed to detect 2-keto-3-deoxy-6-phosphogluconate aldolase. The presence of α-ketoglutarate dehydrogenase suggests that the citrate cycle in both strains is closed. On the other hand, the glyoxylate pathway is not probably functioning, since malate synthase was not detected and the activity of isocitrate lyase was found to be low. The extracts of cells grown in the presence of ammonium sulfate as the source of nitrogen exhibited the activities of glutamine synthetase and glutamate synthase, the enzymes of the glutamate cycle, and of NADPH-dependent glutamate dehydrogenase responsible for the reductive amination of α-ketoglutarate. This suggests that strains Bur 3 and Bur 5 possess two pathways for the primary assimilation of NH_4^+ (the so-called glutamine synthase/glutamate–oxoglutarate aminotransferase system).

Table 1. Effect of pH on the relative content of phospholipids in cells of strain Bur 3 (% of the total)

Phospholipid	pH 7.2	pH 9.0
Phosphatidylcholine	17.5	17.5
Phosphatidylserine	2.5	1.9
Phosphatidylethanolamine$_1$	53.0	43.4
Phosphatidylethanolamine$_2$	2.8	1.5
Phosphatidylglycerol	14.2	26.3
Cardiolipin	10.0	9.4

The degree of DNA–DNA homology between strains Bur 3 and Bur 5 was found to be high (92%), whereas the homology of these strains to *Ancylobacter aquaticus* ATCC 25396^T and *A. vacuolatum* DSM 1277 was considerably lower (30–35 and 28–30%, respectively).

DISCUSSION

Twenty-one bacterial associations isolated from the bottom sediment samples taken from the soda lakes of the southern Transbaikal region were found to be able to grow at pH 9.0–10.0 in a medium with methanol as the sole source of carbon and energy. The associations contained cells of 2–3 different morphotypes. We were unable to isolate particular bacteria from these associations in pure cultures, presumably because of the inability of alkaliphilic aerobic methylotrophs to grow on agar media or because of the requirement of these methylotrophs for some growth factors supplied by their satellites. Nevertheless, we succeeded in isolating two methylotrophic strains, Bur 3 and Bur 5, in pure cultures from the sediment samples taken from lakes

Table 2. Enzymatic activities (in nmol/(min mg protein)) in cell-free extracts of strains Bur 3 and Bur 5

Enzyme	Cofactor	Strain	
		Bur 3	Bur 5
Methanol dehydrogenase	PMS	10	12
Formaldehyde dehydrogenase	PMS	0	36
	NAD^+	0	0
	NAD^+, GSH	127	60
Formate dehydrogenase	PMS	14	23
	NAD^+	42	81
Hydroxypyruvate reductase	NADH	37	32
	NADPH	53	230
Serine–glyoxylate aminotransferase	NAD(P)H	0	0
Malate lyase	ATP, CoA	0	0
Hexulose-3-phosphate synthase		0	0
Ribulose 1,5-bisphosphate carboxylase		157	140
Phosphoribulokinase		28	28
Glucose-6-phosphate dehydrogenase	NAD^+	6	13
	$NADPH^+$	69	38
6-Phosphogluconate dehydrogenase	NAD^+	12	40
	$NADPH^+$	24	80
2-Keto-3-deoxy-6-phosphogluconate aldolase		0	0
Fructose-1,6-bisphosphate aldolase		73	161
6-Phosphofructokinase	ATP	12	20
Pyruvate dehydrogenase	NAD^+	89	146
Citrate synthase		20	10
Isocitrate dehydrogenase	NAD^+	0	0
	NADPH	106	77
α-Ketoglutarate dehydrogenase	NAD^+	42	39
Isocitrate lyase;		6	2
Malate synthas		0	0
Glutamate dehydrogenase	NADH	0	0
	NADPH	21	40
Glutamine synthetase	ADP, Mn^{2+}	365	77
lutamate synthase	NADH	6	40
	NADPH	18	40

Maloe Guzhirnoe and Srednyaya Gorbunka, whose waters have pH 10.0 and 9.2, respectively.

These strains are able to grow at temperatures ranging from 4–6 to 37–42°C. The strains are similar in morphology and in the majority of physiological and biochemical properties. At the same time, strain Bur 3 differs from strain Bur 5 in its ability to grow in an atmosphere of $CO_2 + O_2 + H_2$, to hydrolyze gelatin, and to produce hydrogen sulfide, as well as in the range of antibiotics to which they are susceptible and in the degree of their tolerance to NaCl. The phospholipid composition of cells depends on the pH of the medium: the cellular content of negatively charged phospholipids increases at alkaline pH values. As was suggested by Aono *et al.* [13], the increased content of acidic biopolymers in cells of alkaliphilic bacteria may represent their adaptive response to elevated pH of the medium. In our opinion, the increased content of acidic phospholipids in strain Bur 3 may also serve its adaptation to high pH of the medium and the stabilization of the cell membranes.

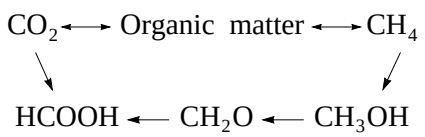
Strains Bur 3 and Bur 5 are facultative methylotrophs with the autotrophic type of C_1-metabolism. Six bacterial genera capable of autotrophic growth on

methanol are presently known: *Ralstonia* (formerly *Alcaligenes*), *Angulomicrobium, Blastobacter, Paracoccus, Xanthobacter*, and *Ancylobacter* (formerly *Microcyclus*). The genus *Ralstonia* represents rod-shaped or coccoid peritrichous cells [14]; the genus *Angulomicrobium* represent tetrahedral and radially symmetric cells reproducing by budding [8]; the genus *Blastobacter* represents nonmotile, budding, yellowish or colorless rod-shaped cells, which are often attached to a substrate and form rosettes [14]; the genus *Paracoccus* represent nonmotile, colorless, short, rod-shaped or coccoid cells reproducing by fission [15]; the genus *Xanthobacter* represents yellow-pigmented, rod-shaped, pleiomorphic cells reproducing by fission [16]; and the genus *Ancylobacter* represents nonmotile, curved or bean-shaped cells, which form circular structures before fission [17, 18].

Phenotypically, strains Bur 3 and Bur 5 are most similar to the species *Ancylobacter aquaticus* [17, 18] and *Ancylobacter vacuolatum* DSM 1277 (formerly *Renobacter vacuolatum* RV[9]). The degree of the DNA–DNA homology of strains Bur 3 and Bur 5 to each other was 92%, whereas 28–35% to the species *A. aquaticus* ATCC 25396^T and *Ancylobacter vacuolatum* DSM 1277. It is generally accepted that the strains whose degree of DNA homology is higher than 70% belong to one species and the strains whose degree of DNA homology is higher than 20% belong to one genus [19]. Therefore, there are grounds to assign strains Bur 3 and Bur 5 to the genus *Ancylobacter.*

Representatives of this genus are common components of the methane-oxidizing microbial associations isolated from marshes and other aqueous ecosystems. For instance, Namsaraev and Zavarzin isolated *A. aquaticus* Z-238 and Z-2434 from a mixed methane-oxidizing culture [20]. Earlier, Kalyuzhnaya *et al.* [7] isolated several methanotrophs from the same bottom sediment samples that were used in this study for the isolation of strains Bur 3 and Bur 5. The close relationship between methanotrophs and methylotrophs may be due to the fact that the latter utilize the intermediates of methane oxidation (methanol, formaldehyde, and formate) that are excreted by methanotrophs into the medium. Furthermore, many strains of the genus *Ancylobacter* are oligotrophic and can utilize hydrogen and other inorganic compounds as electron donors and CO_2 as a carbon source. Such trophic and metabolic flexibility of these chemolithotrophs allows them to live in extreme biotopes [18].

This discussion shows that aerobic methylobacteria can be considered to be active members of the microbial communities of soda lakes. The trophic relationships between (halo)alkaliphilic heterotrophs, oligotrophs, and methylotrophs [1, 7] must also involve aerobic methylobacteria, which provide for the recycling of carbon atoms present in the products of the incomplete oxidation of methane back into the common pool of organic matter in these alkaline ecosystems:



Further thorough investigation of the metabolism and phylogeny of alkaliphilic methylobacteria is of great theoretical and applied importance.

Taxonomic description of *Ancylobacter natronum* sp. nov (na. tro. num. Gr. n. natrun, soda) is named for its source, the soda lake. Cells are gram-negative, nonmotile, bean-shaped, encapsulated, 0.4–0.6 × 1.1–1.4 μm in size, which reproduce by binary fission. Three-day colonies grown on nutrient agar are small (below 1 mm in diameter), rounded, colorless, translucent, with a smooth surface, even edge, and viscous consistency. Requires biotin for growth. Is able to grow at temperatures ranging from 6 to 37°C and at pH ranging from 6.5 to 9.0; optimally grows at 25–29°C and pH 8.0–8.5. Starch and gelatin are hydrolyzed. Nitrates are reduced to nitrites. Indole, ammonia, and hydrogen sulfide are produced. The Voges–Proskauer and methyl red tests are negative. Catalase- and oxidase-positive. Urease is absent. Obligate aerob. When grown aerobically (but not anaerobically) in a medium with glucose, acidifies the medium. Utilizes methanol, glucose, arabinose, galactose, maltose, rhamnose, ribose, raffinose, sucrose, betaine, glutamate, dimethylglycine, serine, adonitol, dulcitol, glycerol, inositol, mannitol, sorbitol, ethanol, acetate, acetamide, malate, and glucuronic acid as sources of carbon and energy. Poorly utilizes formate and formaldehyde. Is able to grow autotrophically in an atmosphere of $CO_2 + O_2 + H_2$. Utilizes $(NH_4)_2SO_4$, KNO_3, glutamate, and aspartate as nitrogen sources. Is unable to grow in the presence of 3% NaCl. Implements the ribulose bisphosphate pathway of C_1-metabolism. NH_4^+ is assimilated by the reductive amination of α-ketoglutarate and in the glutamate cycle. The major ubiquinone is Q_{10}. Phospholipids are dominated by phosphatidylethanolamine and phosphatidylcholine; phosphatidylglycerol, phosphatidylserine, and cardiolipin are present in minor amounts. The relative content of phosphatidylglycerol in cells increases at an elevated pH. The G+C content of DNA is 66.2 mol %, as evaluated from T_m. The type strain *A. natronum* Bur 3^T is isolated from the soda lake Maloe Guzhirnoe in the southern Transbaikal region. The pure culture of this strain has been deposited in the All-Russia Collection of Microorganisms (VKM) as strain VKM B-2242. Strain Bur 5 of this species is isolated from the soda lake Srednyaya Gorbunka in the same region.

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