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EXPERIMENTAL ARTICLES

# Halophilic and Halotolerant Aerobic Methylobacteria from the Technogenic Solikamsk Biotopes

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**Abstract**—Seven strains of moderately halophilic and halotolerant aerobic methylobacteria from the technogenic Solikamsk biotopes (Perm krai, Russia) were isolated in pure cultures and characterized. The isolates were represented by gram-negative and gram-positive (strain 2395B) cells. All the cells were shown to multiply by binary fission without formation of spores or prosthecae. All isolates except strain 2395B were able to oxidize methanol by a classical methanol dehydrogenase. The ribulose monophosphate (RMP) (strain LS), serine (strains S12, S3, 2395A), or ribulose bisphosphate (strains SK15 and S3270) pathways of $C_1$-assimilation were used. In strain 2395B, the key enzymes of the RMP and serine metabolic pathways were determined. Using polyphasic taxonomy, three strains were identified as representatives of the known species: *Arthrobacter protophormiae* 2395B, *Methylophaga thalassica* LS, and *Ancylobacter rudongensis* S3270. Three more strains were identified as members of new species: *Methylopila oligotropha* sp. nov. (strain 2395A$^T$; VKM B-2788$^T$ = CCUG 63805$^T$), *Ancylobacter defluvii* sp. nov. (strain SK15$^T$; VKM B-2789$^T$ = CCUG 63806$^T$), and *Paracoccus communis* sp. nov. (strain S3$^T$; VKM B-2787$^T$ = CCUG 63804$^T$). According to the results of 16S rRNA gene sequencing, the obligately methylotrophic strain S12 had less than 94% similarity with the known genera of the *Proteobacteria* and was probably a representative of a novel genus.

*Keywords*: aerobic methylotrophic bacteria, technogenic biotopes, halotolerant bacteria, halophilic bacteria, new species

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Aerobic methylotrophic bacteria use methanol, methylamine, and other oxidized and substituted derivatives of methane as the carbon and energy sources. Over 40 taxa of methylobacteria have been described [1, 2]. These bacteria were isolated from various biotopes, including those with extreme conditions.

Halophilic and halotolerant methylobacteria are widespread in seawater, soda lakes, saline soils, and other environments [3–5]. Among the first moderately halophilic methylobacteria isolated from the marine ecosystems and showing optimal growth at 3–5% NaCl were the representatives of the genus *Methylophaga*—*M. marina* and *M. thalassica* [6]. Later, novel species of this genus were described [3, 7–11]. "*Methylosulfonomonas methylotropha*" also belongs to the moderately halophilic methylobacteria [12]. Moderately halophilic facultative methylobacteria of the genus *Methylarcula* (*M. marina* and *M. terricola*) were isolated from saline biotopes [13].

Moderately halophilic methylotrophs of the class *Gammaproteobacteria*, *Methylohalomonas lacus* and *Methylonatrum kenyense*, with the optimal growth at 3–5% NaCl, were isolated from the sediments of a hypersaline chloride–sulfate lake of the Kulunda steppe (Altai, Russia) and a soda lake in Kenya [14]. Biodiversity of methylobacteria from technogenic saline ecosystems is, however, poorly studied. The ecosystems of the salt mines and saltworks are potentially rich with halophilic/halotolerant bacteria. In Russian Federation, Solikamsk (Perm krai) is one of the centers of potassium–magnesium salt mining. Solikamsk was founded in the 15th century, close to the salt mines, and in the 17–18th centuries it became the biggest saltwork in Russia. The city is situated within the unique Verkhnekamskiy field of potassium and potassium–magnesium salts (sylvites and carnallites). This field also contains big reserves of chlorine sodium salts (halites). Moreover, within the city on the bank of Usolka river there is the Lyudmilinskaya salt well drilled over a century ago. Specific conditions of enhanced salinity have been maintained in this salt well for a long time, resulting probably in selective pressure, which could promote formation of the microbial communities resistant to high salinity, presumably including methylobacteria. Thus, isolation, identification, and analysis of diverse methylobacteria from saline technogenic biotopes are important tasks.

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The goal of the present work was to determine the physiological, biochemical, and taxonomical characteristics of the new halophilic and halotolerant strains of aerobic methylobacteria isolated from the technogenic biotopes of Solikamsk.

## MATERIALS AND METHODS

**Subjects of the study.** Bacteria isolated in 2009 from the soil, silt, and water samples collected within the territory of the Solikamsk potash mining departments nos. 1, 2, and 3 (the complex for mining and processing of potash fertilizers, Sylvinite Co., Solikamsk, Perm krai, Russia) were used in the study. Physicochemical parameters of the samples were determined in situ using selective electrodes of the Ecotest 120 portable meter (Econics, Russia).

The enrichment and pure cultures were obtained on the K medium with 3% NaCl (wt/vol) and 1% (vol/vol) methanol, as described [5]. Purity of the cultures was confirmed by the homogeneity of the colonies on agar media containing methanol or glucose/peptone, as well as by the light and electron microscopy.

The reference strains for DNA–DNA hybridization were collection type strains *Methylophaga thalassica* VKM B-2057$^T$ (=DSM 5690$^T$), *Ancylobacter rudongensis* DSM 17131$^T$ (=AS1.1761$^T$), *Paracoccus denitrificans* VKM B-1320$^T$ (=DSM 413$^T$), and *Arthrobacter protophormiae* VKM Ac-2104 (=DSM 20168$^T$).

**Determination of the physiological and biochemical properties of the isolates.** For the studies of the colonies, cell morphology, and cell motility, the strains were grown on agar (Difco, United States) K medium containing 1% of $CH_3OH$ (vol/vol). The ability of the isolates to produce indole was determined using the Salkowski reagent in the K medium containing methanol, with $(NH_4)_2SO_4$ replaced by $KNO_3$ (1 g/L) and 0.1% (wt/vol) L-tryptophan [15]. Strain S12, which did not use $KNO_3$ as the source of nitrogen, was grown in the K medium with the concentration of $(NH_4)_2SO_4$ decreased to 0.5 g/L.

To determine the ability of the isolates to use various organic compounds as carbon and energy sources, the tested compound was added to the mineral medium (0.3% wt/vol) instead of methanol. The medium was inoculated with the cell suspension washed off the K agar medium and incubated for 14 days at optimal temperature on a shaker. Volatile substances were added at a concentration of 0.5% (vol/vol).

Additionally, to determine the range of the used substrates and to reveal some biochemical properties of the strains, API tests were used (Biomerieux, France), according to the manufacturer's recommendations.

Halotolerance of the isolates was determined in the liquid K medium with NaCl concentrations from 0 to 20%. Starch and gelatin hydrolysis, as well as oxidase and catalase activities, were determined on the cultures grown on K agar medium as described [16]. Capacity for autotrophic growth was determined in the vials with the gas phase containing $H_2 : O_2 : CO_2$ (7 : 2 : 1).

Requirement for vitamins was studied on the K medium containing thiamin, biotin, or $B_{12}$ (50 μg/L). The control medium did not contain vitamins. Antibiotic resistance was determined using the disks (Bioanalyse, Turkey) [17].

Electron microscopy of the intact cells and ultrathin sections was carried out by the standard methods [18]. Enzyme activity was determined as described previously [3, 19].

**Chemotaxonomic analysis.** Phospholipid composition of the cells was determined using thin-layer chromatography [20].

For the analysis of fatty acids composition, bacteria were grown under optimal conditions, and at the exponential growth phase 30 mg of the biomass was collected. Fatty acids composition was determined using an AT-5850/5973 Agillent Technologies chromato-mass-spectrometer (United States) [20].

**DNA isolation and analysis.** DNA was isolated and purified according to Marmur [21]. Nucleotide composition was defined by thermal denaturation using a Beckman DU-8B spectrophotometer (United States) with the heating rate 0.5°C/min. *Escherichia coli* K-12 DNA was used as a standard. DNA–DNA homology of the isolates and the reference cultures was determined by DNA–DNA reassociation [22].

For amplification of the fragments of 16S rRNA gene, universal bacterial primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-AAGGAAGGTGATCCAGCTCGT-3') were used [23]. PCR amplification was carried out using an MJ Mini DNA thermocycler (BioRad, United States) as follows: initial denaturing at 96°C for 3 min, 27 cycles at 95°C for 40 s, at 58°C for 40 s, at 72°C for 50 s, and the final cycle at 72°C for 4 min. The PCR reaction mixture (30 μL) consisted of 0.5 μM of each primer, 1 μL of DNA (10–100 ng), 200 μM of each dNTP, and 1 U of *Taq* DNA polymerase. The PCR products were separated in 1% agarose gel. The PCR products were isolated and purified on low-melting-point agarose using a Wizard SV Gel Kit and PCR Clean-Up System, according to the manufacturer's recommendations (Promega, United States).

For DNA sequencing, the ABI PRISM BigDye™ Terminator v. 3.1 Cycle Sequencing Kit was used with subsequent analysis of the reaction products in an Applied Biosystems 3730 DNA Analyzer.

**Phylogenetic analysis.** Preliminary screening of the sequences of the 16S rRNA genes of the studied strains was carried out using the GenBank database (NCBI),

**Table 1.** Characteristics of aerobic methylobacteria isolated from the Solikamsk technogenic biotopes

| Characteristics | LS | 2395B | S3270 | SK15 | S3 | 2395A | S12 |
|---|---|---|---|---|---|---|---|
| Type of methylotrophy | Restricted facultative | Facultative | | | | | Obligate |
| Growth substrates | | | | | | | |
| Methanol | + | + | + | + | + | + | + |
| Methylamine | + | + | – | – | + | + | + |
| Dimethylamine | – | + | – | – | + | + | – |
| Glucose | – | – | + | + | + | – | – |
| Fructose | + | + | + | + | + | + | – |
| $NO_3$ reduction to $NO_2$ | – | + | + | + | – | – | – |
| Gelatin hydrolysis | – | + | – | – | + | – | – |
| Starch hydrolysis | + | + | + | + | + | + | + |
| Urease | – | – | + | – | – | + | – |
| $C_1$ assimilation pathway | RMP | RMP, serine | RBP | | Serine | | |
| Predominant fatty acids | $C_{16:0}$ | $C_{18:1w7c}$ $C_{19:0\ cyc}$ | $C_{18:1w7c}$ $C_{16:0}$ | $C_{18:1w7c}$ $C_{19:0\ cyc}$ | $C_{18:1w7c}$ $C_{16:0}$ | $C_{18:1w7c}$ $C_{19:0\ cyc}$ | |
| Range of NaCl, % (optimum) | 1–17 (3) | 0.5–3 (1) | 0–6 (1) | 1–10 (1) | 0.5–10.0 (1) | 0.5–5 (1) | 1–17 (3–5) |
| G+C content, mol % | 44 | ND | 66.2 | 65 | 64.5 | 67 | 60.5 |
| Phylum | *Gammaproteobacteria* | *Actinobacteria* | *Alphaproteobacteria* | | | | |

Note: ND stands for not determined.

the BLAST network server [http://ncbi.nlm.nih.gov]. For more precise determination of the phylogenetic position of the isolates, the 16S rRNA nucleotide sequences were aligned with the reference sequences of the most closely related prokaryotes using the CLUSTAL W program [http://www.genebee.msu.su/clustal]. The rooted phylogenetic tree was constructed using the neighbor-joining technique (NEIGHBOR) implemented in the TREECON package [24]. Evolutionary distance was calculated as the number of 100 nucleotide substitutions. Statistical significance of the branching order was determined by bootstrap analysis of 100 alternative trees using the TREECON software package.

## RESULTS AND DISCUSSION

**Morphology of the isolates.** The studied isolates were represented by gram-negative (strains LS, S3, S12, SK15, S3270, and 2395A) and gram-positive (strain 2395B) strains. Strain S3270 had curved, almost bean-shaped cells, while strain S3 was represented by very short, almost spherical cells. The cells of strain LS were motile due to one polar flagellum. The cells of other isolates were nonmotile, long (LS, S12, and SK15) or short (2395A, and 2395B) rods. The cells of S12 and S3 had a polysaccharide capsule. At the third day of growth on the K agar medium with 0.5% methanol at 29°C, the cells formed round colonies with smooth edge, smooth surface, and concave profile. The colonies were homogenous, colorless, white, or grey, transparent (LS, SK15, S3270) or opaque (S3, S12, 2395A, 2395B). Spores or prosthecae were not formed. The colonies of strain 2395A on the agar medium had a yellowish color. All the isolates multiplied by binary fission.

**Cultural, physiological, biochemical, and chemotaxonomic characteristics.** All strains grew in liquid medium containing methanol without pigment formation or cell aggregation. Additional growth factors were necessary for the strains LS ($B_{12}$) and S12 ($B_{12}$, biotin). All strains were oxidase- and catalase-positive. They used methanol as the source of carbon and energy. The strains LS, S3, S12, 2395A, and 2395B used also methylamine. The strains S3270 and SK15 did not grow on methylamine. Dimethylamine was used by 2395A, 2395B, and S2 (Table 1). In addition to ammonium nitrogen, all strains except S12 used $KNO_3$. The strains S3, 2395A, 2395B, SK15, and S3270 grew on polycarbon substrates (facultative methylobacteria), strain LS grew only on fructose (restricted facultative methylotroph), while strain S12 was able to use only single-carbon substrates (obligate methylotroph). The strains S3 and 2395B grew on glucose, maltose, and rhamnose; strains S3, SK15, 2395B, and S3270 grew on sorbitol; strains S3, SK15, and S3270, on mannitol; strains SK15, 2395A, and 2395B, on malate. The strains SK15 and S3270 were

**Table 2.** Enzyme activity (nmol $min^{-1}$ $mg^{-1}$ of protein) in the extracts of methanol-grown cells

| Enzyme | Cofactor | Strain | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | S3 | S12 | 2395A | 2395B | LS | SK15 | S3270 |
| Methanol dehydrogenase | PMS | 170 | 317 | 25 | 0 | 90 | 64 | 235 |
| Formaldehyde dehydrogenase | PMS | 28 | 28 | 36 | 38 | 8 | 25 | 23 |
| | $NAD^+$ | 44 | 10 | 2 | 15 | 0 | 25 | 34 |
| | $NAD^+$, GSH | 180 | 6 | 2 | 10 | 0 | 126 | 97 |
| Formate dehydrogenase | PMS | 26 | 23 | 150 | 171 | 5 | 27 | 143 |
| | $NAD^+$ | 5 | 20 | 108 | 5 | 35 | 24 | 149 |
| Hydroxypyruvate reductase | NADPH | 700 | 165 | 443 | 2132 | 0 | 98 | 2 |
| | NADH | 147 | 100 | 172 | 910 | 0 | 13 | 0 |
| Serine-glyoxylate amynotransferase | NADPH | 600 | 251 | 23 | 70 | 0 | 0 | 0 |
| | NADH | 236 | 100 | 58 | 123 | 0 | 0 | 0 |
| Gexulosophosphate synthase | – | 0 | 0 | 0 | 395 | 79 | 0 | 0 |
| Ribulose bisphosphate carboxylase | – | 0 | 0 | 0 | 0 | 0 | 155 | 107 |
| Isocitrate lyase | – | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| 2-Keto-3-desoxy-6-phosphogluconate aldolase | – | 0 | 0 | 0 | 0 | 58 | 0 | 0 |
| Fructose-1,6-bisphosphate aldolase | – | 15 | 16 | 23 | 52 | 0 | 12 | 7 |

Note: PMS stands for phenazine methosulfate.

capable of autotrophic growth in the atmosphere $H_2 : O_2 : CO_2$.

The strains 2395B, SK15, and S3270 demonstrated ability for nitrate reduction. Soluble starch was hydrolized by all studied isolates, while gelatin was hydrolyzed only by 2395B and S3 (Table 1). The studied strains did not form ammonia or hydrogen sulfide when grown on the test media. The strains LS, S3, S12, 2395A, and 2395B synthesized indoles (0.5–1.7 μg/mL).

Although strains 2395A, 2395B, S3, SK15, and S3270 had the same growth optimum (~1% NaCl), their halotolerance was significantly different. Thus, strain 2395B survived at 3% NaCl, while strains S3270 and 2395A grew at 6% NaCl. At the same time, S3 and SK15 were able to grow at 10% NaCl and, hence, were halotolerant. Since strains LS ans S12 grew at concentrations of NaCl up to 17%, their growth optimum being 3–5% NaCl, they may be considered moderately halophilic bacteria.

**Metabolic characteristics.** In the cell extracts of the analysed strains, the activities of methanol dehydrogenases (except 2395B), as well as of formaldehyde and formate dehydrogenase, were revealed (Table 2). Strains LS and 2395B showed activity of hexulose phosphate synthase, the key enzyme of the ribulose monophosphate pathway. Strains LS and 2395B exhibited the activities of 2-keto-3-deoxy-6-phosphogluconate aldolase (KDPG) and fructose-1,6-bisphosphate aldolase (FBPA), respectively. Therefore, strain LS used the KDPG pathway and strain 2395B used the FBPA variant of the RMP pathway. Notably, strain 2395B was also shown to possess the key enzymes of the serine pathway, hydroxypyruvate reductase and serine glyoxylate aminotransferase. This may be due to the fact that strain 2395B isolate used simultaneously both the RMP and serine pathways of $C_1$ metabolism. In strains S3, S12, and 2395A, while the activities of the key enzymes of the serine pathway, hydroxypyruvate reductase and serine glyoxylate aminotransferase, were detected, the activity of isocitrate lyase was not found. Thus, these strains may use the ICL-negative variant of the serine pathway. In the cells of SK15 and S3270, activity of ribulose bisphosphate carboxylase was found. This is the key enzyme of the ribulose bisphosphate pathway. However, they did not possess hexulose phosphate synthase and serine-glyoxylate aminotransferase.

**Chemo- and genotaxonomic characteristics.** In the composition of fatty acids of the studied strains

**Table 3.** Cellular fatty acids composition of the cells of investigated methylobacteria (% of the total)

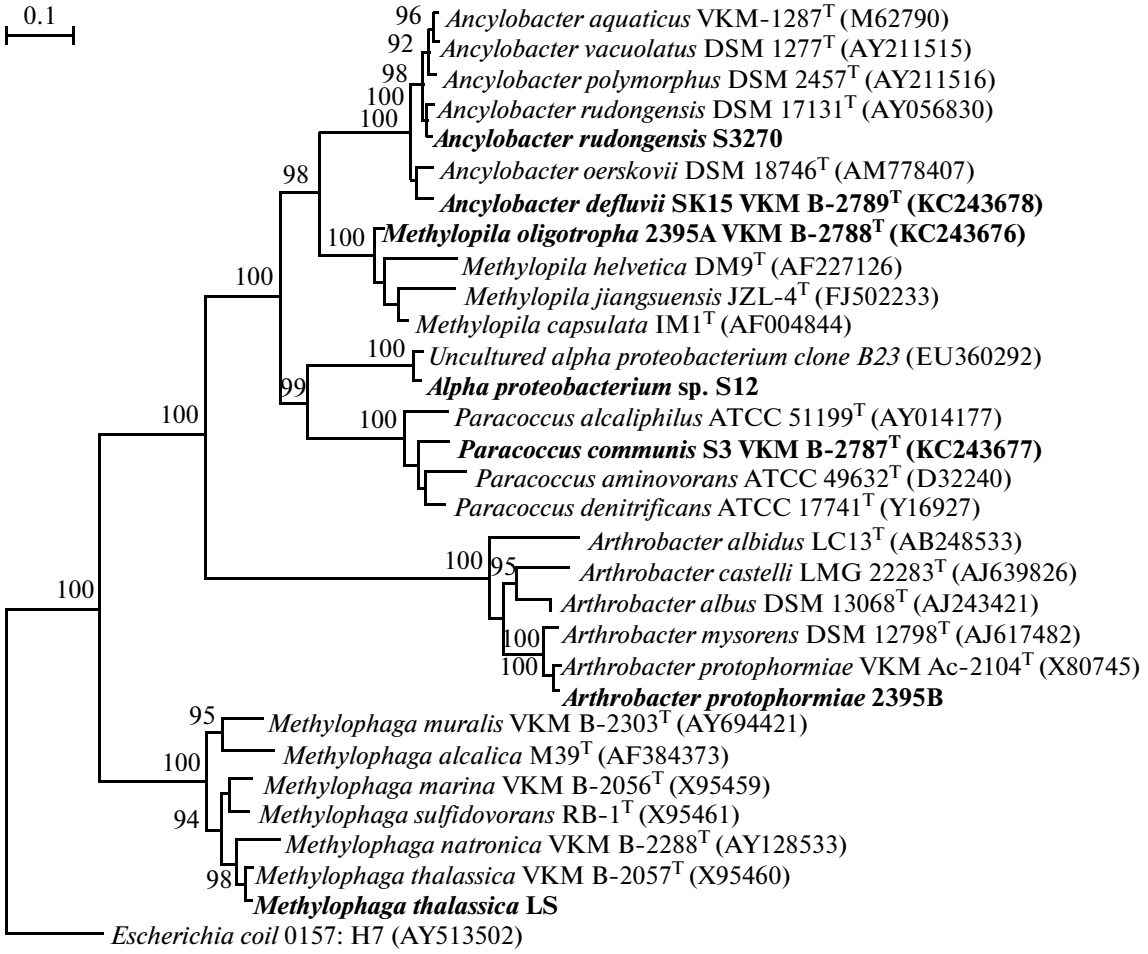
| Fatty acids | Hydroxy acids | | | | | |
|---|---|---|---|---|---|---|
| | S3 | S12 | SK15 | S3270 | 2395A | 2395B |
| $C_{12:1}$ | 0.6 | – | – | – | – | – |
| $C_{15:0}$ | 0.3 | – | – | – | – | – |
| $C_{i15}$ | – | – | – | – | – | 0.3 |
| $C_{a15}$ | – | – | – | – | – | 1.4 |
| $C_{16:0}$ | 14.0 | 0.6 | 7.4 | 6.2 | 2.4 | 2.0 |
| $C_{16:1\omega7c}$ | 0.5 | – | 0.3 | 1.0 | 0.4 | 0.5 |
| $C_{16:1\omega9}$ | – | – | – | 0.6 | – | – |
| $C_{17:0}$ | 2.6 | – | – | – | 1.0 | 0.6 |
| $C_{17:1}$ | 0.3 | – | – | – | – | – |
| $C_{18:0}$ | 3.3 | 17.4 | 2.0 | 4.0 | 2.0 | 1.3 |
| $C_{18:2}$ | – | – | – | 0.4 | 5.1 | 5.6 |
| $C_{18:1\omega7c}$ | 74.0 | 36.7 | 77.0 | 78.0 | 68.0 | 64.5 |
| $C_{18:1\omega9}$ | – | – | – | 1.0 | 0.6 | 0.5 |
| $C_{19:0\ cyc}$ | 1.7 | 28.7 | 12.3 | 5.4 | 10.0 | 12.4 |
| $C_{19:1}$ | – | – | – | – | 0.4 | 0.3 |
| $C_{20:0}$ | – | 1.7 | – | – | – | – |
| $C_{20:1\omega9}$ | – | 5.2 | – | – | 0.7 | 0.8 |
| $C_{11\text{-Me }18:1}$ | – | 4.8 | 1.0 | 3.3 | 2.0 | 2.0 |
| Hydroxy acids | | | | | | |
| 3-OH $C_{10:0}$ | 0.7 | – | – | – | – | – |
| 2-OH $C_{19:1}$ | – | – | – | – | 2.0 | 2.0 |
| 2-OH $C_{21:1}$ | – | – | – | – | 3.8 | 4.7 |
| 3-OH $C_{24:0}$ | – | 1.7 | – | – | – | – |
| 3-OH $C_{25:0}$ | – | 1.4 | – | – | – | – |
| 3-OH $C_{26:0}$ | – | 1.6 | – | – | – | – |
| 3-OH $C_{14:0}$ | 0.5 | 0.2 | – | 0.1 | 1.0 | 0.7 |
| 3-OH $C_{18:0}$ | 0.2 | – | – | – | 0.6 | 0.4 |
| 3-OH $C_{18:1}$ | 0.5 | – | – | – | – | – |
| 3-OH $C_{20:1}$ | 0.8 | – | – | – | – | – |

(Table 3) *cis*-11-octadecenoic acid prevailed (36.7–78.0%). For strain S3, hexadecanoic acid (14%) was typical, while in S12, cyclopropane nonadecanoic (28.7%) and octadecanoic (17.4%) acids were found. The strains S23270 and SK15 contained cyclopropane nonadecanoic (5.4 and 12.3%, respectively) and hexadecanoic (6.2 and 7.4%, respectively) acids. Strains 2395A and 2395B synthesized cyclopropane nonadecanoic (10.0 and 12.4%) and octadecadienoic (5.1 and 5.6%) acids. The highest amount of hydroxy acids (2-hydroxy nonadecenoic and 2-hydroxyuncosanoic acids) was typical of the strains 2395A and 2395B. Strain S3 was found to contain 3-hydroxy decanoic acid (0.7%).

Predominant phospholipids were phosphatidylethanolamine and phosphatidylcholine, while phosphatidylglycerol and diphosphatidylglycerol were also detected. In strain 2395B, phosphatidylglycerol, phosphatidylcholine, and phosphatidylethanolamine were the dominant phospholipids.

Phylogenetic position of the strains derived from analysis of the 16S rRNA gene sequences is shown in the figure.

Strain LS was found to have high similarity of the 16S rRNA gene nucleotide sequences (95.2–99.9%) to members of the genus *Methylophaga*. Strain LS exhibited maximal similarity (99.9%) to *M. thalassica* VKM B-2057$^T$ (=DSM 5690$^T$), and DNA–DNA homology was 100%. Therefore, strain LS belongs to this species (figure). The DNA G+C content of this




Phylogenetic position of the strains based on comparative study of the 16S rRNA gene sequences. The scale corresponds to 10 nucleotide substitutions for every 100 nucleotides (evolutionary distance). The root was determined by insertion of *Escherichia coli* 0157: H7 sequence (AY513502) as the outgroup. The numerals show statistical significance of the branching order determined using bootstrap analysis of 100 alternative trees.

strain was 44 mol % ($T_m$), corresponding to that of *M. thalassica* VKM B-2057$^T$.

According to sequencing of the 16S rRNA gene, gram-positive strain 2395B was related to *Arthrobacter protophormiae* VKM Ac-2104$^T$ (=DSM 20168$^T$) (100% of similarity).

The strains SK15 and S3270 exhibited maximal similarity (97.8–99.0%) of the 16S rRNA gene sequences to the representatives of the genus *Ancylobacter*. The similarity between the sequences of strains SK15 and S3270 was 98.4%. According to the DNA heat denaturation, the DNA G+C content of strains SK15 and S3270 was 65.9 and 66.2 mol % ($T_m$), respectively. Strain S3270 demonstrated 99.8% similarity to *A. rudongensis* DSM 17131$^T$ and 99.4%, to *A. aquaticus* VKM B-1287$^T$. High ratio of DNA–DNA relatedness between *A. rudongensis* DSM 17131$^T$ (78%) and S3270 was shown. Thus, strain S3270 belongs to this species.

Based on the obtained results, three strains were identified as the known species: *Arthrobacter protophormiae* 2395B, *Methylophaga thalassica* LS, and *Ancylobacter rudongensis* S3270.

Strain SK15 exhibited 98.3% similarity to *A. oerskovii* DSM 18746$^T$ and *A. rudongensis* DSM 17131$^T$, 98.0% similarity to *A. vacuolatus* VKM B-1381$^T$ (=DSM 1277$^T$) and *A. aquaticus* VKM B-1287$^T$ (=DSM 101$^T$). The level of DNA–DNA homology of strain SK15 with *A. rudongensis* and *A. oerskovii* was 29–32%. Hence, SK15 represented a novel species for which the name *Ancylobacter delfluvii* sp. nov. was proposed.

Phylogenetic analysis of the sequences of the 16S rRNA genes revealed 97.8% similarity between strain S3 and *Paracoccus denitrificans* VKM B-1320$^T$ (=DSM 413$^T$) and 96.5% similarity between it and *Paracoccus aminovorans* VKM B-2140$^T$ (=DSM 8537$^T$). According to DNA–DNA hybridization, strain S3 and the type strain of *P. denitrificans* had 42%

of homology. The DNA G+C content of S3 was 64.5 mol % ($T_m$). Therefore, S3 strain could be considered as the new species with the proposed name *Paracoccus communis* sp. nov.

The similarity of 16S rRNA gene sequences of strain 2395A and the representatives of the genus *Methylopila* was the 98.5% with *Methylopila capsulata* IM1$^T$ (VKM B-1606$^T$) and 98.2% with *Methylopila jiangsuensis* JZL-4$^T$ (=VKM B-2555$^T$). DNA–DNA hybridization of the new isolate with *M. capsulata* type strain revealed 56% homology. Thus, strain 2395A was a novel species, with the proposed name *Methylopila oligotropha* sp. nov.

Sequencing of the 16S rRNA gene revealed that strain S12 belonged to the phylum *Alphaproteobacteria*, although homology with the known bacterial genera was less than 94% except for the cloned sequence B23 (EU360292) retrieved from Pacific ocean water (99.5%). This strain is presumably the representative of a novel species.

Thus, seven strains of aerobic methylobacteria were isolated from the high-salinity technogenic biotopes of Solikamsk. Two strains (LS and S12) appeared to be moderate halophiles with growth optimum at 3–5% NaCl, five isolates were halotolerant with growth optimum at 1% NaCl (although they were able to survive at NaCl concentrations of 3–10%). According to their nutrition type, we revealed the obligate (S12), restricted facultative (LS), and typical facultative (S3, S3270, SK15, 2395A, and 2395B) methylobacteria.

Among the isolated cultures, we found the strains implementing the following metabolic pathways: ribulose monophosphate pathway (LS), serine pathway (S12, S3, and 2395A), and ribulose bisphosphate pathway (S3270 and SK15). Interestingly, the facultatively methylotropic strain 2395B demonstrated the activities of the key enzymes for two $C_1$-assimilation pathways (RMP and serine). This may help this strain to adapt more easily to the permanently fluctuating environment.

Chemotaxonomic characteristics were typical of the genera *Paracoccus, Ancylobacter*, and *Methylopila*: $Q_{10}$ was the dominant ubiquinone; the dominant fatty acids were *cis*-11-octadecenoic, hexadecanoic, and cyclopropane nonadecanoic acids; in strain S3, 3-hydroxydecanoic acid was found, which is common among the *Paracoccus* species.

*Ancylobacter defluvii* SK15 exhibited the halotolerance unusual for the genus *Ancylobacter*: the strain grew at 10% NaCl, while the growth of other strains of the genus was suppressed at 5% NaCl.

Among the halotolerant representatives of the genus *Paracoccus*, which grew (similar to *Paracoccus communis* S3) at 9% NaCl, the following species were described: *P. rhizosphaerae, P. stylophorae, P. saliphilus, P. homiensis*, and *P. seriniphilus*. However, the growth optimum of these strains was shifted to higher salinity (3–8% NaCl) [25].

Similar to most strains of the genus *Methylopila, Methylopila oligotropha* 2395A did not grow at 3% NaCl. *M. oligotropha* 2395A, unlike the type strain of *M. capsulata*, did not utilize glycerol and was not able to reduce nitrates to nitrites.

Other phenotypical characteristics and differences from members of the corresponding genera are given in the descriptions of the novel species.

Therefore, the taxonomic position of seven methylotrophic isolates from the saline technogenic biotopes of Solikamsk is represented by various genera of the phyla *Alphaproteobacteria, Gammaproteobacteria*, and *Actinobacteria*, including three novel species of methylobacteria and one novel genera of alphaproteobacteria.

### *Methylopila oligotropha* sp. nov.

*O.li.go.tro'pha., oligos*, a little, several, *trophos*, feeding, *oligotropha* uses a limited range of polycarbon substrates.

The cells are gram-negative, motile, short asporogenous rods (1.0 × 1.4–2.0 μm), and are monotrichs which multiply by binary fission. Colonies formed on mineral agar medium with methanol are white (yellowish on the medium with peptone), opaque, with concave profile and smooth edge. Growth occurs at 16–40°C, pH 5.0–10.0, and at 0.5–5% NaCl. Growth optimum is at 29°C, pH 8.0–8.5. They do not need vitamins or other growth factors. Starch, but not gelatin, is hydrolyzed. They are oxidase-, catalase-, and urease-positive. The organism is strictly aerobic. Nitrates are not reduced to nitrites. A restricted facultative methylotrophs uses the ICL-negative variant of the serine $C_1$ metabolic pathway. Methanol, methylamines, and potassium gluconate, but not glucose, fructose, sucrose, arabinose, melibiose, mannose, maltose, sorbitol, inositol, mannitol, malate, glycerol, sodium citrate, or amygdaline are utilized. Ammonia salts, nitrates, and methylamine may be used as nitrogen sources. The organism exhibits β-galactosidase, ornithin decarboxylase, and arginine dehydrolase activities. Predominant fatty acids are *cis*-11-octadecenoic (18:1ω7c), 68% and cyclopropane nonadecanoic (19:0cyc), 10% acids. Major phospholipids are phosphatidylethanolamine and diphosphatidylglycerol. The dominant ubiquinone is $Q_{10}$. The DNA C+G content is 67 mol % ($T_m$).

The cells are sensitive to tetracycline, neomycin, streptomycin, kanamycin, penicillin, and gentamycin; they are resistant to oxacillin, novobiocin, lincomycin, nalidixic acid, erythromycin, and chloramphenicol.

The type strain 2395A$^T$ was isolated from soil on the territory of salt mines at Solikamsk, Russia and was deposited to the All-Russian and Swedish Collections of Microorganisms (VKM B-2788$^T$ = CCUG 63805$^T$). The 16S rRNA gene sequence was deposited to GenBank under accession number KC243676.

***Paracoccus communis* sp. nov.**

*Com'mu.nis., communis*, common, without significant differences.

The organism has gram-negative, spherical, nonmotile, asporogenous cells of 0.7 × 0.75 μm, single or in pairs, forming polysaccharide capsules. It multiples by binary fission and forms opaque colonies, creamy-white on the medium with methanol the colonies and yellowish on the medium with peptone. It grows at 16–40°C, pH 6.0–9.5. The growth optimum is 29°C, pH 7.5. The isolate is halotolerant, surviving up to 10% NaCl.

The organism is oxidase- and catalase-positive, capable of denitrification. Growth occurs on a wide range of substrates (methanol, methylamines, glucose, mannose, rhamnose, sodium citrate, sodium aspartate, sorbitol, mannitol, pyruvate, amygdaline, and arginine), but not on arabinose, fructose, melibiose, xylose, malate, serine, or alanine. Ammonia and nitrates may be used as nitrogen sources. It uses the serine pathway of $C_1$ metabolism, and exhibits ornithine decarboxylase activity. Lysin decarboxylase, β-galactosidase, and β-glucosidase activities were not determined. Starch and gelatin are hydrolyzed.

The dominant ubiquinone is $Q_{10}$. Predominant fatty acids are *cis*-11-octadecenoic (18:1ω7c), 74% and hexadecanoic (16:0), 14% acids. The DNA C+G content is 64.5 mol.% ($T_m$).

The cells are sensitive to novobiocin, streptomycin, kanamycin, chloramphenicol, and neomycin; resistant to oxacillin, gentamycin, tetracycline, erythromycin, lincomycin, penicillin, and nalidixic acid.

The type strain S3 was isolated from surface wastes of a sludge warehouse of Solikamsk, Russia and was deposited to the All-Russian and Swedish Collections of Microorganisms (VKM B-2787$^T$ = CCUG 63804$^T$). The 16S rRNA gene sequence was deposited to GenBank under accession number KC243677.

***Ancylobacter defluvii* sp. nov.**

*De.flu'vi.i., defluvii*, stagnant water, pollution, isolated from polluted water close to the sludge warehouse.

The organism has short, almost spherical gram-negative nonmotile rods of 0.6–0.7 × 0.8–1.0 μm, single or in pairs; it divides by binary fission. On the medium containing methanol or peptone, it forms semitransparent grayish-white colonies. The organism is strictly aerobic. The organism is halotolerant: it grows at up to 10% NaCl with the optimum at 1%. Growth optimum is 29°C, pH 7.0; it is oxidase- and catalase-positive. Nitrates are reduced to nitrites. Starch is hydrolyzed, but gelatin is not liquefied. Indole is not produced from tryptophan. Arginin dehydrolase, β-galactosidase, lysin decarboxylase, and ornithindecarboxylase activities were not determined. Ammonium salts are used as the source of nitrogen. The organism is capable of autotrophic growth in the atmosphere of $H_2 : O_2 : CO_2$. It is a cultative methylotroph, growing on methanol using the ribulose bisphosphate pathway of $C_1$ metabolism. It grows on glucose, fructose, mannitol, sorbitol, malate, and gluconate; it does not grow on methylamine, dimethylamine, sucrose, mannose, maltose, rhamnose, melibiose, sodium citrate, inositol, or amygdaline. The predominant fatty acids are *cis*-11-octadecenoic (18:1w7c), 77% and cyclopropane nonadecanoic (19:0cyc), 12.3% acids. The dominant ubiquinone is $Q_{10}$. The major phospholipids are phosphatidylcholine, phosphatidylethanolamine, and diphosphatidylglycerol. The DNA C+G content is 65.1 mol % ($T_m$).

The cells are sensitive to kanamycin, neomycin, and streptomycin; they are resistant to penicillin, tetracycline, gentamycin, oxacillin, novobiocin, erythromycin, chloramphenicol, nalidixic acid, and lincomycin.

The type strain SK15 strain was isolated from the wastes of a salt mine in Solikamsk, Russia and was deposited to the All-Russian and Swedish Collections of Microorganisms (VKM B-2789$^T$ = CCUG 63906$^T$). The 16S rRNA gene sequence was deposited to GenBank under accession number KC243678.

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