ISSN 0026-2617, Microbiology, 2018, Vol. 87, No. 5, pp. 662–671. © Pleiades Publishing, Ltd., 2018.
Original Russian Text © N.V. Doronina, E.N. Kaparullina, A.A. Chemodurova, Yu.A. Trotsenko, 2018, published in Mikrobiologiya, 2018, Vol. 87, No. 5, pp. 541–550.

EXPERIMENTAL ARTICLES

Paracoccus simplex sp. nov., a New Methylamine-Utilizing Facultative Methylotroph

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Received February 22, 2018

Abstract—A facultative methylotroph, strain F5^T, which uses methylamine and a broad spectrum of polycarbon substrates as carbon and energy sources, was isolated from silt of a freshwater lake in the southern Moscow region. The cells were gram-negative, coccoid, non-spore-forming, nonmotile, colorless, reproducing by binary fission and possessing a capsule. The organism was mesophilic, neutrophilic, not halophilic, oxidase- and catalase-positive, and capable of nitrate reduction to nitrite. Methylamine was oxidized by amine dehydrogenase and via the icl$^-$ serine pathway of C_1 metabolism, as was indicated by activities of hydroxypyruvate reductase and serine-glyoxylate aminotransferase and by the absence of hexulosephosphate synthase and ribulose bisphosphate carboxylase. Predominant fatty acids were $C_{18:1\omega7c}$ (72.3%) and $C_{16:0}$ (11.6%). Phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylethanolamine were the dominant phospholipids. The G + C DNA content was 65.8 mol % (T_m). Q_{10} was the dominant ubiquinone. Strain F5^T exhibited high similarity of the 16S rRNA gene sequences with *Paracoccus* strains: *P. aminovorans* JCM7685^T = VKM B-2140^T (98.0%), *P. huijuniae* FLN-7^T (97.9%), and *P. limosus* NB88^T (97.5%). However, the level of DNA-DNA relatedness between the strains F5^T and *P. aminovorans*^{T} was only 21 ± 3%. Based on the data obtained, strain F5^T was identified as a new *Paracoccus* species with proposed name *Paracoccus simplex* sp. nov. (= VKM B-3226^T = CCUG 71989^T).

Keywords: Paracoccus simplex sp. nov., facultative methylotroph

DOI: 10.1134/S0026261718050077

The genus *Paracoccus* belongs to the family *Rhodobacteraceae*, the order *Rhodobacterales*, the class *Alphaproteobacteria*, and was originally described by Davis et al. (1969); the description was subsequently emended (Katayama et al., 1995; Liu et al., 2008). The new information was generalized and presented in the chapters of the 3rd and 4th editions of "Prokaryotes" (Kelly et al., 2006a; 2006b; Pujalte et al., 2014). At present, the genus includes 48 species. Paracocci are gram-negative cocci or short rods, mostly nonmotile, although the members possessing one polar flagellum (*P. homiensis* DD-R11^T and *P. versutus* ATCC 25364^T) and peritrichous flagellation (*P. carotinofaciens* E-396^T) have been described. Reproduction occurs by binary fission; the colonies of some species are orange due to carotenoid production. The organisms are catalase- and oxidase-positive, the predominant ubiquinone is Q_{10}, although Q_8 is predominant in *P. yeei* CDC G1212^T. Some species are halophilic or halotolerant. Paracocci are aerobic chemoorganotrophs, but some species can utilize nitrate, reducing it to nitrite or molecular nitrogen. The type species *Paracoccus denitrificans* can grow chemolithoautotrophically, using reduced sulfur compounds as substrates or oxidizing hydrogen. Some *Paracoccus* species are facultative methylotrophs and utilize methanol or methylamine as a carbon and energy source but use different C_1-metabolic pathways. In the case of autotrophic growth, CO_2 fixation occurs via the Calvin cycle, and such *Paracoccus* species possess ribulose bisphosphate carboxylase (RuBisCO); other species are devoid of RuBisCO and use the serine pathway of C_1 assimilation (*P. aminophilus*). Methylamine oxidation is catalyzed by methylamine dehydrogenase or occurs via the N-methylglutamate pathway. Thus, different *Paracoccus* species vary substantially in their physiological and biochemical properties.

The work was aimed at physiological and biochemical characterization and identification of a novel isolate of facultative methylotrophic bacteria, strain F5^T, attributed to the genus *Paracoccus*.

MATERIALS AND METHODS

Research subjects. Strain F5^T (VKM B-3226^T = CCUG 71989^T) was isolated from coast silt of a small

freshwater lake in the vicinity of the town of Pushchino (Moscow oblast, Russia). The silt (3 g) was placed into a 750-mL Erlenmeyer flask with 200 mL of K medium with methylamine hydrochloride 0.3% (wt/vol) and incubated at 28°C, 180 rpm, for 3 days. Enrichment and pure cultures were obtained as described (Doronina et al., 2013). Culture purity was tested by light (Nikon Eclipse Ci, Japan) and electron (JEOL JEM-100B, JEOL, Japan) microscopy, as well as by the uniformity of colonies on agar media with methylamine and glucose/peptone. The studies of morphology, chemotaxonomic, physiological, biochemical, and cultural properties, DNA isolation, 16S rRNA gene sequencing, phylogenetic analysis, determination of DNA G + C content and DNA–DNA hybridization were performed as described previously (Doronina et al., 2013). The K medium contained the following (g/L): KH_2PO_4, 2.0; $(NH_4)_2SO_4$, 2.0; NaCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.125; $FeSO_4 \cdot 7H_2O$, 0.002; pH 7.4. Cultivation was also carried out on R2A, TSA, and LB media. *Paracoccus aminovorans* JCM7685^T = VKM B-2140^T was used as a reference culture.

Cell extracts and enzyme activities. The cells were harvested in the exponential phase by centrifugation at 6000 *g*, washed twice with 50 mM Tris-HCl buffer (pH 7.4), and resuspended in the same buffer. The cells were homogenized on a Sonicator S-4000 ultrasonic disintegrator (6 × 1 min, with 1-min breaks) under cooling in ice. Intact cells were separated by centrifugation (14000 rpm for 30 min, 4°C). Supernatants were used for enzymological analysis.

Amine oxidase was assayed by hydrogen peroxide production (Haywood and Large, 1981). The reaction mixture (2 mL) contained: Tris-HCl buffer, pH 7.5, 150 μmol; 10 U horseradish peroxidase, 1 μmol *o*-Dianisidine, 16 μmol methylamine, and the extract. The reaction was started by adding methylamine; the changes in extinction were recorded at 460 nm.

Methylamine dehydrogenase was assayed by DCPIP reduction in the presence of PMS (Meiberg and Harder, 1978). The reaction mixture (2 mL) contained (μmol): K-phosphate buffer, pH 7.5, 150; DCPIP, 0.8; PMS, 4; KCN, 2, methylamine, 7; and the extract. The reaction was started by adding methylamine, and the rate of the changes in extinction was recorded at 600 nm.

N-methylglutamate dehydrogenase was assayed by formaldehyde production measured with the Nash reagent (Nash, 1953). The reaction mixture (2 mL) contained (μmol): K-phosphate buffer, pH 7.5, 100; NAD^+, 0.25; and the extract. The reaction was started by adding sodium N-methyl-L-glutamate (5 μmol).

Formaldehyde and formate dehydrogenases were assayed by DCPIP reduction (Johnson and Quayle, 1964). The reaction mixture (2 mL) contained (μmol): K-phosphate buffer, pH 7.5, 50; DCPIP, 0.075; PMS, 0.5; formaldehyde, 10, or formate, 50; and the extract. The reaction was started by adding the substrate.

HAD$^+$-dependent formaldehyde dehydrogenase was assayed by NAD^+ reduction at 340 nm (Johnson and Quayle, 1964). The reaction mixture (2 mL) contained (μmol): K-phosphate buffer, pH 7.0, 50; NAD^+, 0.25; reduced glutathione, GSH (or without it), 10; formaldehyde, 2; and the extract. The reaction was started by adding formaldehyde.

NAD$^+$-dependent formate dehydrogenase was assayed by NAD^+ reduction at 340 nm (Johnson and Quayle, 1964). The reaction mixture (2 mL) contained (μmol): Tris-HCl buffer, pH 7.5, 50; HAD, 0.25; formate, 50; and the extract. The reaction was started by adding formate.

Hydroxypyruvate reductase was assayed by NAD(P)H oxidation (Blackmore and Quayle, 1970). The reaction mixture (2 mL) contained (μmol): Tris-HCl buffer, pH 7.4, 100; NAD(P)H, 0.5; and the extract. The reaction was started by adding 5 μmol of sodium hydroxypyruvate. In the presence of NADH reductase, the result was corrected for NADH oxidation by the extract without hydroxypyruvate.

L-serine glyoxylate aminotransferase was assayed spectrophotometrically by recording the glyoxylate-dependent formation of hydroxypyruvate from L-serine (Blackmore and Quayle, 1970). The reaction mixture (2 mL) contained (μmol): Tris-HCl buffer, pH 7.5, 100; pyridoxal phosphate, 0.02; NADH, 0.5; glyoxylate, 10; and the extract. The reaction was started by adding 10 μmol of L-serine. The appropriate correction was made for the result in the presence of glyoxylate reductase activity in the extracts.

Ribulose bisphosphate carboxylase was assayed by the radioisotope method by monitoring the dynamics of $NaH^{14}CO_3$ inclusion into the acid-resistant product. The reaction mixture (1 mL) contained (μmol): Tris-HCl buffer, pH 7.6, 100; $MgCl_2$, 2.5; reduced glutathione (GSH), 10; ribulose-1,5-bisphosphatte (sodium salt), 0.25; and the extract; the reaction was started by adding 18 μmol $NaH^{14}CO_3$ (20 μCu). The reaction was carried out at 30°C; 0.02-mL samples were taken during 20 min with 2-min intervals, applied to Whatman GF/F glass microfiber paper squares, fixed with 0.05 mL of 6 N HCl, and dried; then the radioactivity was measured.

Hexulose phosphate synthase was assayed spectrophotometrically (Ferenci et al., 1974) by $NADP^+$ reduction in the glucose-6-phosphate dehydrogenase- and glucose phosphate isomerase-mediated reaction. The reaction mixture (2 mL) contained (μmol): K-phosphate buffer, pH 7.0; $MgCl_2$, 8; $NADP^+$, 0.5;

rabbit muscle phosphoglucose isomerase, 1.68 μmolar units; glucose-6-phosphate dehydrogenase (type 7, Sigma), 0.15 μmolar units; ribose-5-phosphate; and the extract. The reaction was started by adding 5 μmol of formaldehyde.

Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were assayed by $NADP^+$ or NAD^+ reduction (Korndberg and Horecker, 1955). The reaction mixture (2 mL) contained (μmol): K-phosphate buffer, pH 7.5, 150; $MgCl_2$, 10; $NAD(P)^+$, 1.0; glucose-6-phosphate sodium salt or 6-phosphogluconate sodium salt, 10; and the extract. The reaction was started by adding the appropriate substrate.

Fructose-1,6-bisphosphate aldolase was assayed with the coupling enzyme glycerophosphate dehydrogenase (van Dijken and Quayle, 1977). The reaction mixture (2 mL) contained (μmol): Tris-HCl buffer, pH 7.5, 100; $CoCl_2$, 2; NADH, 0.5; glycerophosphate dehydrogenase, 0.36 U; and the extract. The reaction was started by adding 2 μmol of fructose-1,6-bisphosphate.

2-Keto-3-deoxy-6-phospohgluconate aldolase was assayed with the coupling enzyme lactate dehydrogenase (Wood, 1971). The reaction mixture (2 mL) contained (μmol): imidazole buffer, pH 8.0, 50; 6-phosphogluconate (sodium salt), 10; NADH, 0.5; $MgCl_2$, 5; dithiotreitol, 2; lactate dehydrogenase from pig muscle (Reanal), 5 U; and the extract. The reaction was started by adding 6-phosphogluconate. The control was the reaction mixture without 6-phosphogluconate.

Isocitrate lyase was assayed spectrophotometrically by glyoxylate phenylhydrazone formation at 324 nm (Dixon and Kornberg, 1959). The reaction mixture (2 mL) contained (μmol): K-phosphate buffer, pH 6.8, 150; $MgCl_2$, 10; phenylhydrazine, 6.5; cysteine-HCl, 4; and the extract. The reaction was started by adding 5 μmol of potassium isocitrate.

Glutamate dehydrogenase was assayed in the reaction mixture (2 mL) containing (μmol): Tris-HCl buffer, pH 7.5, 100; NH_4Cl, 80; NAD(P)H, 0.5; and the extract. The reaction was started by adding 10 μmol of α-ketoglutarate.

Glutamate synthase was assayed by NAD(P)H oxidation (Meers and Tempest, 1970). The reaction mixture (2 mL) contained (μmol): Tris-HCl buffer, pH 7.6, 100; α-ketoglutarate, 10; NADH or NADPH, 0.25; and the extract. The reaction was started by adding 25 μmol of glutamine.

Glutamine synthetase was assayed by the modified colorimetric method (Elliott, 1955) in the γ-glutamine transferase reaction. The reaction mixture (7.52 mL) contained (μmol): imidazole buffer, pH 7.15; $MgCl_2$, 0.27; sodium arsenate, 12.5; ADP, 0.18; hydroxylamine, 20; and the extract. The reaction was started by adding 20 μmol of L-glutamine; the mixture was incubated for 10 min at 30°C, followed by the addition of 0.6 mL of solution containing (g/L): 55 g of $FeCl_3$, 20 g of trichloroacetic acid, and 21 mL of HCl. The mixture without L-glutamine was used as a control. The formed precipitate was removed by centrifugation and the absorption was measured at 540 nm. The value of 0.63 absorbance unit corresponded to 1 μmol/mL of γ-glutamyl hydroxamate (the reaction product).

Optical measurements were performed on a Shimadzu UV-1700 spectrophotometer (Japan) in a thermostated cuvette at 30°C. Enzyme activities were expressed as a number of nanomoles of transformed substrate or formed product in 1 min per 1 mg of protein. Specific activity was assayed with the amount of protein providing linear dependence of the rate on the enzyme concentration. The calculations were made with the following molar extinction coefficients, $\mu mol^{-1}\ cm^{-1}$: NAD(P)H (at 340 nm), 6.22; DCPIP (at 600 nm), 21.9; *o*-Dianisidine, (at 460 nm) 28.8. Radiometric measurements were made with a LS6500 Multi-Purpose Scintillating Counter (Beckman Coulter, United States) in the mixture containing 4 g of 2,5-diphenyloxazole (PPO) and 0.05 g of 1,3-bis(5-phenyloxazole-2yl)benzene (POPOP) in 1 L of toluene.

Protein assay was performed by the Lowry method (Lowry et al., 1951) using Sigma reagents (United States).

RESULTS AND DISCUSSION

Isolate morphology. The cells of strain $F5^T$ were gram-negative, nonmotile, coccoid, 0.4–0.7 × 0.5–0.9 μm (Figs. 1a, 1b), with a capsule. Did not form spores and pigment, reproduced by binary fission. The colonies on R2A agar medium were circular, white, glossy, transparent, 2–3 mm in diameter (day 3, 29°C), with wavy edges and viscous consistency.

Cultural, physiological, biochemical, and chemotaxonomic properties. Strain $F5^T$ grew in the liquid K medium with methylamine without cell aggregation, did not form pigment. It was aerobic, catalase- and oxidase-positive, needed no vitamins or other growth factors. Grew in the temperature range from 17 to 37°C (optimal 28–30°C) and pH 5.5–8.0 (optimal pH 7.0–7.5). Used the following broad range of substrates as the carbon and energy sources: methylamine, trimethylamine, ethanol, glucose, galactose, fructose, maltose, mannose, trehalose, ribose, sucrose, succinate, pyruvate, α-ketoglutarate, malate, acetate, betaine, alanine, serine, valine, inositol, mannitol, acetamide, and glucuronic acid. No growth occurred on methanol, dimethylamine, formate,

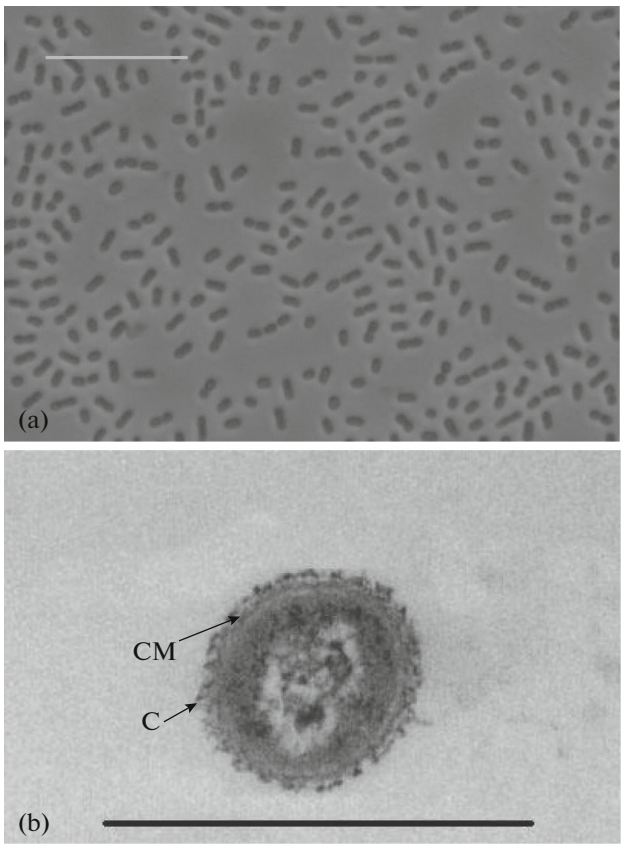

Fig. 1. Morphology of the F5^T cells grown on methylamine: phase contrast microscopy, scale bar, 10 μm (a); ultrathin section of the cells showing the gram-negative cell wall and the presence of an external capsule (b). The arrows show: cytoplasmic membrane (CM) and capsule (C). Scale bar is 1 μm.

dimethyl sulfoxide, dimethylformamide, dichloromethane, arabinose, lactose, xylose, glutamate, aspartate, and in the $H_2/CO_2/O_2$ gas mixture. Ammonium and nitrates were used as nitrogen sources. The isolate was capable of nitrate reduction. Starch was hydrolyzed. Gelatinase was not present. The optimal NaCl concentration in the medium was 0.05%; growth was observed up to 2.5% NaCl and was completely absent at 3.5% NaCl.

The strain was sensitive to the following antibiotics (μg/disc): ampicillin (10), gentamycin (10), lincomycin (2), nalidixic acid (30), novobiocin (30) and streptomycin (10). Resistant to kanamycin (30), neomycin (30) and erythromycin (15). The fatty acid composition of the cells included $C_{18:1\omega7c}$ (72.3%), $C_{16:0}$ (11.6%), $C_{18:0}$ (3.2%), and $C_{19:0\ cyc}$ (1.9%) fatty acids. The predominant phospholipids were phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine (Fig. 2). The predominant ubiquinone was Q_{10}.

Metabolic characteristics. The results of enzymological analysis of the cells grown on methylamine are presented in Table 1. It was established that strain F5^T had a high methylamine dehydrogenase activity demonstrating the activity with phenazine methosulfate (PMS). The product of this reaction (formaldehyde) was oxidized to formate by NAD^+-glutathione-dependent dehydrogenase and then to CO_2 by NAD^+-dependent formate dehydrogenase. Amine oxidase and N-methylglutamate dehydrogenase were absent.

Hydroxypyruvate reductase and serine-glyoxylate aminotransferase activities were detected. 3-Hexulose phosphate synthase and ribulose-1,5-bisphosphate carboxylase activities were not detected. Isocitrate lyase activity was very low. This enzyme was probably not associated with C_1 metabolism. Consequently, strain F5^T implementd the isocitrate lyase-negative (icl^-) variant of the serine pathway. Isocitrate dehydrogenase was $NADP^+$-dependent. Primary involvement of ammonium nitrogen was implemented via the

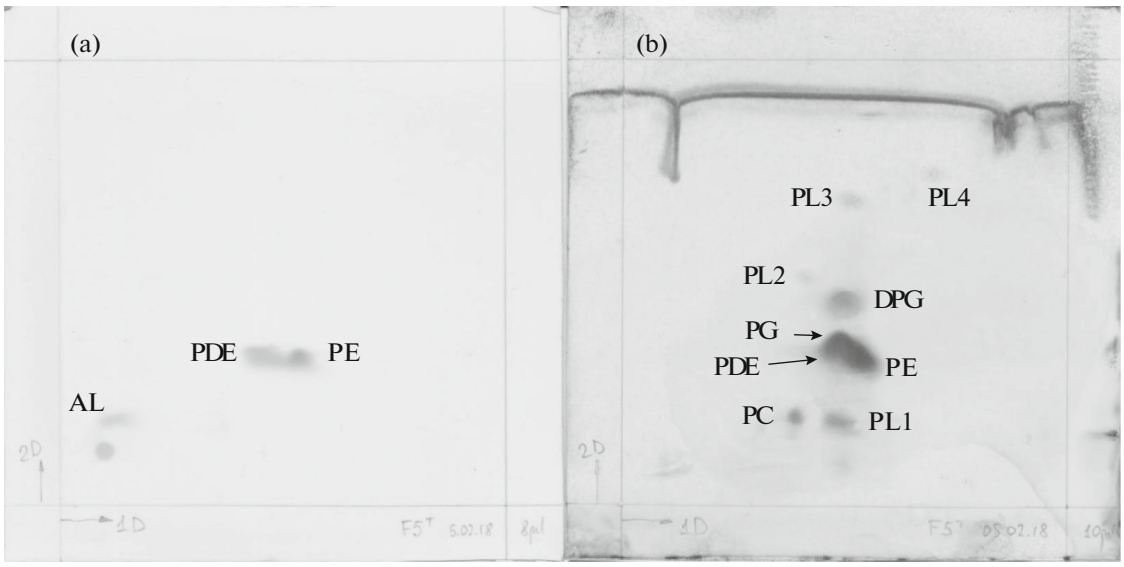

Fig. 2. Two-dimensional chromatography of the polar lipids of strain *Paracoccus simplex* F5^T. Direction 1, chloroform–methanol–water (65 : 25 : 4); direction 2, chloroform–methanol–acetic acid–water (85 : 12 : 15 : 4). The assay of amino-containing lipids with 0.2% ninhydrin in acetone (a); detection of phosphorus-containing lipids with molybdenum blue (b). Designations: phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), diphosphatidylethanolamine (PDE), diphosphatidylglycerol (DPG), unidentified phospholipids (PL1–4), unidentified amino lipids (AL).

glutamate pathway and by glutamate dehydrogenase. The enzyme activities of carbohydrate metabolism were very low.

Phylogenetic analysis. The 16S rRNA gene sequencing of the studied strain F5^T (1405 bp) showed the high level of similarity with representatives of the genus *Paracoccus*: 98.0% with *P. aminovorans* JCM 7685^T (D32240), 97.9% with *P. huijuniae* FLN-7^T (EU725799), and 97.5% with *P. limosus* NB88^T (HQ336256) (Fig. 3). The DNA G + C content in strain F5^T was 65.8 mol %. The level of DNA–DNA homology between strain F5^T and *P. aminovorans* JCM 7685^T was only 21 ± 3%. Strain F5^T was also phylogenetically characterized using *mauA,* the gene encoding the small subunit of methylamine dehydrogenase. The phylogenetic position of strain F5^T based on the comparison of MauA amino acid sequences is shown on Fig. 4. Strain F5^T had the maximum similarity (96–100%) in the MauA protein with representatives of the genus *Paracoccus* and some bacteria of other genera: *Methylobacterium* (90–92%), *Methylopila* (89–91%), and *Methylophaga* (77–78%).

The results of polyphasic analysis indicated that strain F5^T was a novel species of the genus *Paracoccus*, with the proposed name of *Paracoccus simplex* sp. nov.

The differentiating characteristics of *P. simplex* sp. nov. and of the phylogenetically closest members of the genus *Paracoccus* are given in Table 2.

P. simplex sp. nov., in contrast to *P. aminovorans* JCM 7685^T, has a tryptophan deaminase, utilizes the broader range of polycarbon substrates, and does not grow on dimethylamine and dimethylformamide; in contrast to *P. huijuniae* FLN-7^T, it cannot grow at 42°C and utilize methanol. The studied strain F5^T differs from *P. limosus* NB88^T in gluconate and maltose utilization. Moreover, unlike the latter two species, it cannot grow at 4% NaCl in the medium.

Description of *Paracoccus simplex* sp. nov. *Paracoccus simplex* sp. nov. (*sim'plex. L. adj. simplex, simple*). The cells are gram-negative, non-motile, non-spore-forming rods 0.4–0.7 × 0.5–0.9 μm, capsule-forming, reproducing by binary fission. The colonies on the R2A medium are round, white, glossy, transparent, 2–3 mm in diameter with a wavy edge and a viscous consistency. Grows at a temperature of 17 to 37°C with an optimum at 28–30°C and at pH 5.5 to 8.0, optimally at pH 7.0–7.5. Does not grow at 3.5% NaCl in the medium. Grows on R2A, TSA, and LB agar. Obligate aerobic bacterium. Does not grow under anaerobic conditions and does not use nitrate as an electron acceptor. Oxidase and catalase positive, urease-negative. Nitrates are reduced to nitrites. Negative for

Table 1. The activities of enzymes of the primary and intermediate metabolism in cell extracts of the strain F5^T grown on methylamine*

Enzyme	Cofactor	Activity, nmol/(min mg protein)
Methylamine dehydrogenase	PMS	111
	NAD^+	0
Aminoxidase		0
N-methylglutamate dehydrogenase		0
Formaldehyde dehydrogenase	PMS	0
	NAD^+	37
	NAD^+GSH	800
Formate dehydrogenase	PMS	0
	NAD^+	11
Hydroxypyruvate reductase	NADH	147
	NADPH	0
Serine-glyoxylate transaminase	NADH	120
	NADPH	34
Isocitrate lyase		1
3-Hexulose phosphate synthase		0
Ribulose-1,5-bisphosphate carboxylase		0
Glucose-6-phosphate dehydrogenase	NAD^+	0
	$NADP^+$	6
6-Phosphogluconate dehydrogenase	NAD^+	0
	$NADP^+$	14
Fructose-1,6-bisphosphate aldolase		15
2-Keto-3-deoxy-6-phosphogluconate aldolase		0
Isocitrate dehydrogenase	NAD^+	8
	$NADP^+$	177
Glutamate dehydrogenase	NADH	47
	NADPH	362
Glutamate synthase	NADH	26
	NADPH	45
Glutamate synthetase	ATP, Mn^{2+}	15

* Average values of the results of three independent experiments (standard error not exceeding ±5%); PMS, phenazine methosulfate; GSH, reduced glutathione.

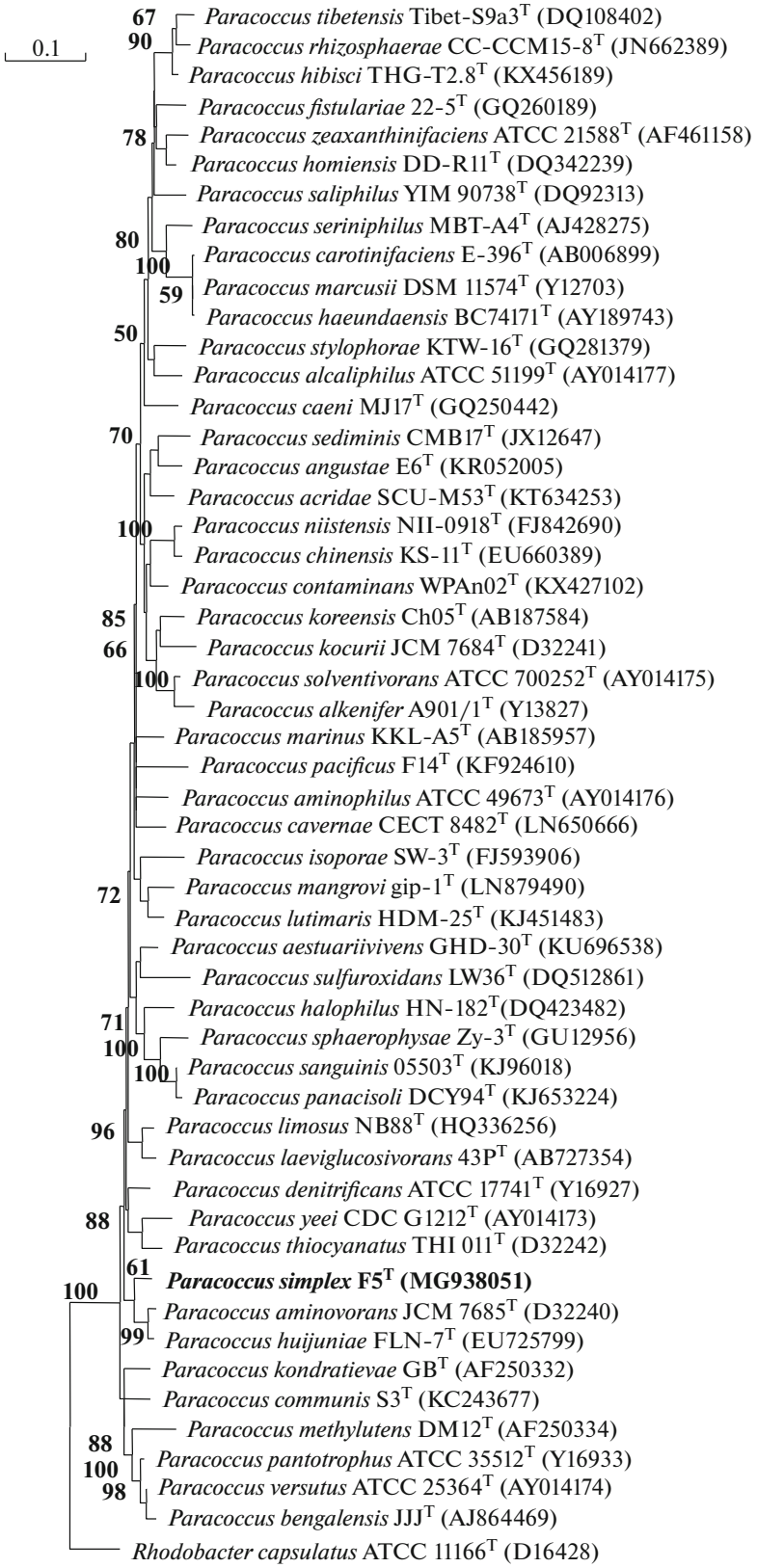

Fig. 3. The phylogenetic position of strain F5^T based on the results of comparative analysis of the 16S rRNA gene sequences. The scale corresponds to 10 nucleotide substitutions per every 100 nucleotides (evolutionary distance). The neighbor-joining method was used. The root was determined by including the *Rhodobacter capsulatus* ATCC 11166^T (D16428) sequence as an outgroup.

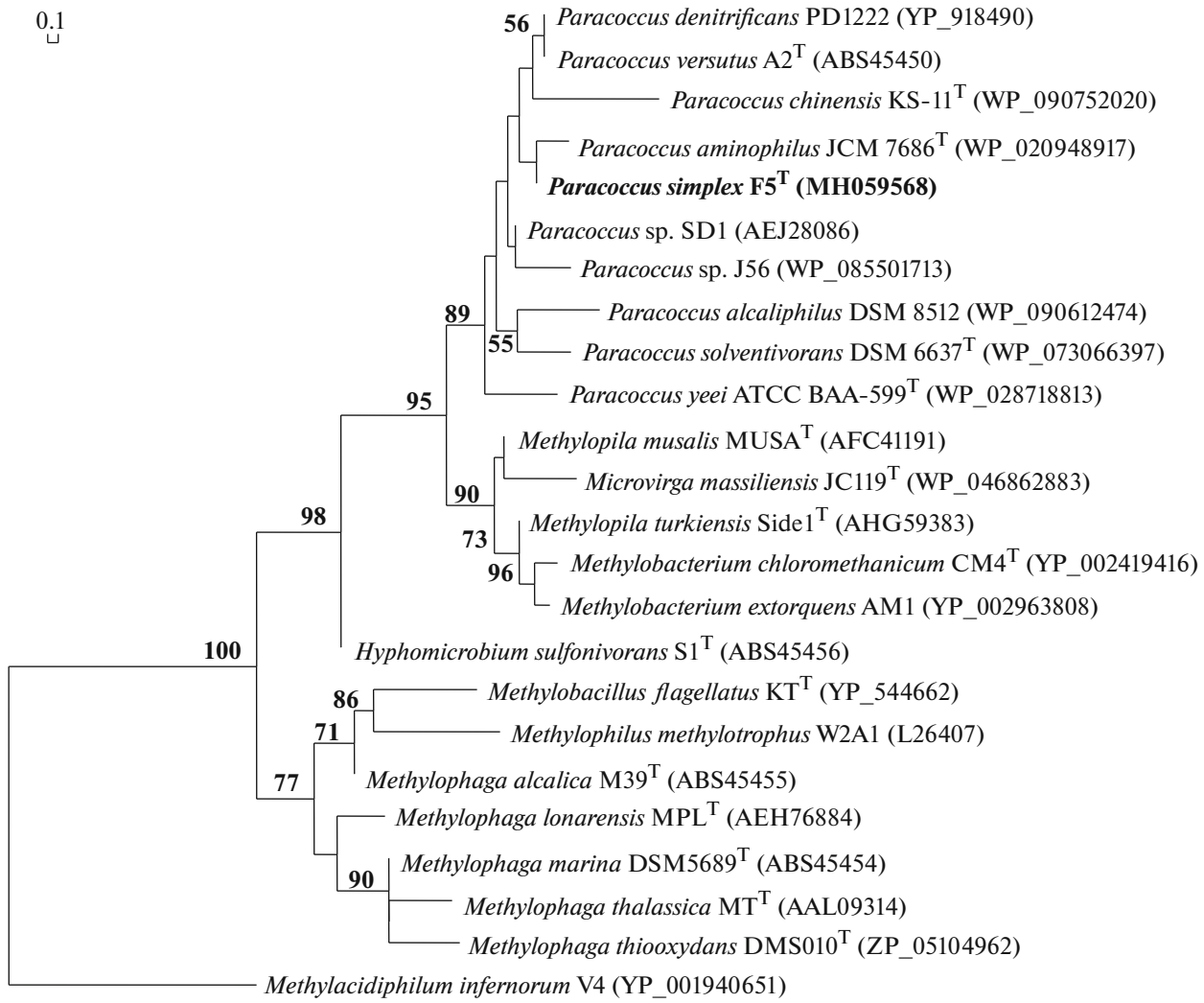

Fig. 4. The phylogenetic position of the strain F5^T based on comparison of amino acid sequences of the MauA protein. The scale corresponds to 10 amino acid substitutions per every 100 amino acids (evolutionary distance). The root was determined by including the sequence of *Methylacidiphilum infernorum* V4 (YP_001940651) as an outgroup. The numbers show the statistical confidence of branching order determined by the "bootstrap" analysis of 100 alternative trees.

β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, but positive for tryptophandeamine, produces indole and acetoin. Grows on methylamine, trimethylamine, ethanol, glucose, galactose, fructose, maltose, mannose, trehalose, ribose, sucrose, succinate, pyruvate, α-ketoglutarate, malate, acetate, betaine, alanine, serine, valine, inositol, mannitol, acetamide, glucuronic acid. Does not grow on methanol, dimethylamine, formate, dimethyl sulfoxide, dimethylformamide, dichloromethane, arabinose, lactose, xylose, glutamate, aspartate, and also in the $H_2/CO_2/O_2$ atmosphere. No vitamins or other growth factors are required. Implements the isocitrate lyase-negative (icl$^-$) variant of the serine pathway. The dominant respiratory quinone is Q_{10}. In the fatty acid composition of cells, more than 70% is $C_{18:1\omega7c}$. There are also $C_{16:0}$ (11.6%), $C_{18:0}$ (3.2%) and C_{19cyc} (1.9%) acids. Phospholipid composition of the cells includes phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylethanolamine. The DNA G + C content of the type strain F5^T is 65.8 mol %. The 16S rRNA and *mauA* genes sequences of the strain F5^T were deposited in the GenBank/EMBL/DDBL under accession numbers MG938051 and MH059568, respectively. The type strain, F5^T (= VKM B-3226^T = CCUG 71989^T), was isolated from a coastal freshwater lake in the vicinity of Pushchino, Moscow Region (Russia).

Table 2. Comparative characteristics of *Paracoccus simplex* F5^T and phylogenetically related species: 1, *P. simplex* F5^T sp. nov.; 2, *P. aminovorans* JCM 7685^T (Urakami et al., 1990); 3, *P. huijuniae* FLN-7^T (Sun et al., 2013); and 4, *P. limosus* NB88^T (Lee, M.J. and Lee, S.S, 2013)

Characteristic	1	2	3	4
Cell size, μm	0.4–0.7 × 0.5–0.9	0.5–0.9 × 0.9–2.0	0.4–0.5 × 0.7–1.1	0.8–0.9 × 0.9–1.1
Colony color	White	White-pale yellow	Pale yellow	Creamy-white
Growth temperature range (optimum), °C	17–37 (28–30)	20–37 (30–37)	4–42 (28)	20–37 (30)
pH range (optimum)	5.5–8.0 (7.0–7.5)	6.0–9.0 (6.5–8.0)	5.0–8.0 (7.0)	6.0–9.0 (7.0)
Growth at 4% NaCl (wt/vol)	–	–	+	+
Tryptophan deaminase	+	–	–	NA
Carbon sources:				
Acetate	+	+	–	+
Gluconate	+	+	–	–
D-glucose	+	–	+	+
D-ribose	+	–	–	+
D-mannose	+	–	+	v
Arabinose	+	–	+	+
Maltose	+	–	–	–
Sucrose	+	–	–	+
L-alanine	+	+	–	+
L-serine	+	v	–	+
Methanol	–	–	+	NA
Methylamine	+	+	+	NA
Dimethylamine	-	+	NA	NA
Trimethylamine	+	+	NA	NA
Fatty acids				
$C_{17:0}$	–	–	+	+
$C_{19:0\ cyc}$	+	+	+	–
DNA G + C (mol %)	65.8	67.0	66.4	66.4

NA, not assayed; v, variable.

REFERENCES

Blackmore, M.A. and Quayle, J.R., Microbial growth on oxalate by a route not involving glyoxylate carboligase, *Biochem. J.*, 1970, vol. 118, pp. 53–59.

Davis, D.H., Doudoroff, M., Stanier, R.Y., and Mandel, M., Proposal to reject the genus *Hydrogenomonas*: taxonomic implications, *Int. J. Syst. Evol. Microbiol.*, 1969, vol. 19, pp. 375–390.

Dixon, G.H. and Kornberg, H.L., *Assay methods for key enzymes of the glyoxylate cycle*, *Proc. Biochem. Soc.*, 1959, p. 3.

Doronina, N.V., Kaparullina, E.N., and Trotsenko, Y.A. *Methylopila musalis* sp. nov., an aerobic facultatively methylotrophic bacterium isolated from banana fruit, *Int. J. Syst. Evol. Microbiol.*, 2013, vol. 63, pp. 1847–1852.

Elliott, W.H., Glutamine synthesis: glutamic acid + ATP + ammonia → glutamine + ADP + phosphate. *Methods in Enzymology,* Colowick, S.P. and Kaplan, N.O., Eds., New York: Academic, 1955, vol. 2, pp. 337–342.

Ferenci, T., Strom, T., and Quayle, J.R., Purification and properties of 3-hexulose phosphate synthase and phospho-3-hexuloisomerase from *Methylococcus capsulatus, Biochem. J.*, 1974, vol. 144, pp. 477–486.

Haywood, G.W. and Large, P.J., Microbial oxidation of amines, *Biochem. J.*, 1981, vol. 199, pp. 187–201.

Johnson, P.A. and Quayle, J.R., Microbial growth on C_1-compounds. Oxidation of methanol, formaldehyde and formate by methanol-grown *Pseudomonas* AM1, *Biochem. J.*, 1964, vol. 93, pp. 281–290.

Katayama, Y., Hiraish, A., and Kuraishi, H., *Paracoccus thiocyanatus* sp. nov., a new species of thiocyanate-utilizing facultative chemolithotroph, and transfer of *Thiobacillus versutus* to the genus *Paracoccus* as *Paracoccus versutus* comb. nov. with emendation of the genus, *Microbiology* (UK), 1995, vol. 141, pp. 1469–1477.

Kelly, D., Rainey, F., and Wood, A., The Genus *Paracoccus*, in *The Prokaryotes*, vol. 5, 3rd edn, Proteobacteria—alpha and beta subclasses, Rosenberg, E., Stackebrandt, E., Thompson, F., Lory, S., and DeLong, E., Eds., Berlin: Springer, 2006a, pp. 232–249.

Kelly, D.P., Euzéby, J.P., Goodhew, C.F., and Wood, A.P., Redefining *Paracoccus denitrificans* and *Paracoccus pantotrophus* and the case for a reassessment of the strains held by international culture collections, *Int. J. Syst. Evol. Microbiol.*, 2006b, vol. 56, pp. 2495–2500.

Kornberg, A. and Horecker, B.L., Glucose-6-phosphate dehydrogenase, *Methods in Enzymology*, 1955, vol. 1, pp. 323–327.

Lee, M.J. and Lee, S.S., *Paracoccus limosus* sp. nov., isolated from activated sludge in a sewage treatment plant, *Int. J. Syst. Evol. Microbiol.*, 2013, vol. 63, pp. 1311–1316.

Liu, Z.P., Wang, B.J., Liu, X.Y., Dai, X., Liu, Y.H., and Liu, S.J., *Paracoccus halophilus* sp. nov., isolated from marine sediment of the South China Sea, China, and emended description of the genus *Paracoccus* Davis 1969, *Int. J. Syst. Evol. Microbiol.*, 2008, vol. 58, pp. 257–261.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., Protein measurement with the Folin phenol reagent, *J. Biol. Chem.*, 1951, vol. 193, pp. 265–275.

Meers, J.L., Tempest, D.W., and Brown, C.M., Glutamine (amide): α-oxoglutarate aminotransferase oxidoreductase (NADP) an enzyme involved in biosynthesis of glutamate by some bacteria, *J. Gen. Microbiol.*, 1970, vol. 64, pp. 187–194.

Meiberg, J.B.M. and Harder, W., Aerobic and anaerobic metabolism of trimethylamine, dimethylamine and methylamine in *Hyphomicrobium* sp. X, *J. Gen. Microbiol.*, 1978, vol. 106, pp. 265–276.

Nash, T., The colorimetric estimation of formaldehyde by means of the Hantzsch reaction, *Biochem. J.*, 1953, vol. 55, pp. 416–421.

Pujalte, M.J., Lucena, T., Ruvira, M.A., Arahal, D.R., and Macián, M.C., The *Paracoccus* Group, in *The Prokaryotes—Alphaproteobacteria and Betaproteobacteria*, 4rd edn., Rosenberg, E., DeLong, E., Lory, S., Stackebrandt, E., and Thompson, F., Eds., Berlin: Springer, 2014, p. 460.

Sun, L.N., Zhang, J., Kwon, S.W., He, J., Zhou, S.G., and Li, S.P., *Paracoccus huijuniae* sp. nov., an amide pesticide-degrading bacterium isolated from activated sludge of a wastewater biotreatment system, *Int. J. Syst. Evol. Microbiol.*, 2013, vol. 63, pp. 1132–1137.

Urakami, T., Araki, H., Oyanagi, H., Suzuki, K.I., and Komagata, K., *Paracoccus aminophilus* sp. nov. and *Paracoccus aminovorans* sp. nov., which utilize N,N-dimethylformamide, *Int. J. Syst. Bacteriol.*, 1990, vol. 40, pp. 287–291.

Van Dijken, J.P. and Quayle, J.R., Fructose metabolism in four *Pseudomonas* species, *Arch. Microbiol.*, 1977, vol. 114, pp. 281–286.

Wood, W.A., Assay of enzymes representative of metabolic pathways, *Methods Microbiol.*, 1971, vol. 6A, p. 421.

Translated by E. Makeeva