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EXPERIMENTAL ARTICLES

Methylopila turkiensis sp. nov., a New Aerobic Facultatively Methylotrophic Phytosymbiont

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Abstract—A new facultative methylotroph, strain Side1^T, was isolated from the phyllosphere of *Bougainvillea* sp. L. The isolate is represented by rod-shaped, aerobic gram-negative asporogenous bacteria which divide by binary fission. Methanol and mono- and trimethylamine were utilized, as well as a limited spectrum of polycarbon substrates, while methane and dichloromethane were not used. Growth occurred at pH 6.0–9.0 with the optimum at pH 7.0 within the temperature range from 20 to 40°C (optimum at 28–30°C) and 0–2.5% NaCl in the medium. The predominant fatty acids were *cis*-11-octadecenoic (C18:1ω7c), 11-methyl-octadecenoic (C18:ω7c11Me), and stearic (C18:0) acids. Phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, and diphosphatidylglycerol were the dominant phospholipids. Q_{10} was the dominant ubiquinone. The isolate oxidized methanol and methylamine by the appropriate dehydrogenases. The isocitrate lyase-negative variant of the serine pathway was used. Ammonium assimilation involved glutamate dehydrogenase and the glutamate cycle (glutamate synthase and glutamine synthetase). The strain synthesized indole and siderophores; it solubilized insoluble phosphates. The DNA G+C content (T_m) was 65.4 mol %. While the nucleotide sequence of the 16S rRNA gene of strain Side1 exhibited high similarity to those of *Methylopila* species (*M. musalis* MUSA^T and *M. capsulata* IM1^T), DNA–DNA homology with these cultures was 32–37%. The results obtained supported classification of strain Side1^T as a new species *Methylopila turkiensis* sp. nov. (VKM B-2748^T = DSM 27566^T).

Keywords: Methylopila turkiensis sp. nov., aerobic methylobacteria, phytosymbiosis

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Methanol, methylated amines, and other C_1 compounds are known as the natural products of plant metabolism and may act as carbon and energy sources for aerobic methylobacteria which, in contrast to methanotrophs, do not grow on methane [1]. Over 50 taxa of aerobic methylobacteria isolated from natural and anthropogenic biotopes have been described as of now [1, 2]. Previously it has been shown that the plant phyllosphere and rhizosphere are colonized by methylobacteria of different taxonomic groups, which supply plants with different bioactive compounds [1]. According to www.bacterio.org, the genus *Methylopila* includes five species of aerobic, gram-negative, nonpigmented facultative methylobacteria implementing the isocitrate lyase-negative variant of the serine pathway for the assimilation of C_1 compounds: *Methylopila capsulata* IM1^T [3], *M. helvetica* DM9^T [4], *M. jiangsuensis* JZL-4^T [5], *M. musalis* MUSA^T [6], and *M. oligotropha* 2395A^T [7]. Most members of this genus were isolated from activated sludge and soils, and only *Methylopila musalis* MUSA^T was isolated from the fruit of the banana *Musa paradisiaca* L. [6].

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The goal of this work was the taxonomic, physiological, and biochemical characterization of the new aerobic methylobacterial strain isolated from the phyllosphere of *Bougainvillea* sp. L.

MATERIALS AND METHODS

Research subjects. The strain Side1^T was isolated from the leaves of the plant *Bougainvillea* sp. L. collected in the vicinity of the town of Side (Turkey). Weighed portions (5 g) of the leaves were put into an Erlenmeyer flask (750 mL) with 200 mL of the K medium and 0.5% (vol/vol) methanol. The K medium contained the following (g/L): KH_2PO_4, 2.0; $(NH_4)_2SO_4$, 2.0; NaCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.1; $FeSO_4 \cdot 7H_2O$, 0.002; pH 7.4. The enrichment and pure cultures were obtained as described [6]. The culture purity was tested by the light and electron microscopy, as well as by homogeneity of the colonies grown on agar media with methanol and glucose/peptone.

The type strains of the genus *Methylopila* (*M. jiangsuensis* JZL-4^T (=VKM B-2555^T = DSM 22718^T = ATCC 05406^T), *M. capsulata* IM1^T (=VKM B-1606^T = ATCC 700716^T), *M. helvetica* DM9^T (=VKM B-2189^T =

CIP106788^T), *M. musalis* MUSA^T (=VKM B-2646^T = DSM 24986^T = CCUG 61696^T), and *M. oligotropha* 2395A^T (=VKM B-2788^T = CCUG 63805^T) were used as the reference strains.

Cultural, physiological, and biochemical properties of the isolate. Colony growth, morphology, and motility of strain Side1^T were studied on solid K medium (Difco agar, United States, 2%). The ability of the isolate to reduce nitrates was analyzed in a liquid medium, where ammonium nitrogen was replaced by KNO_3 (1 g/L) after the first, second, and third day of incubation. Starch hydrolysis was assessed by the reaction with Lugol's iodine solution after growing the culture on the agarized K medium with 0.2% (wt/vol) soluble starch.

The 1% (wt/vol) solution of tetramethyl-*p*-phenylenediamine dihydrochloride was used to detect the presence of oxidase. Catalase activity was detected by applying 3% hydrogen peroxide solution on a streak culture grown on agar medium.

The growth temperature range was determined by growing the culture in liquid K medium with methanol in sealed flasks on a rotary shaker (120 rpm) at 4–43°C. The growth of the isolate at different methanol concentrations (0.1–7.0%, vol/vol), salinity (0–3% NaCl), and pH values was studied in the K medium. The pH values were adjusted by adding 1 M NaOH and 5 N HCl; the pH optimum was determined by specific growth rate of the strain in the exponential phase at initial pH values of 5.0–10.0.

The ability of the isolate to utilize various organic compounds as carbon and energy sources was studied as follows: prior to inoculation, 0.05–0.3% (wt/vol) of the tested substrate was added to the mineral medium instead of methanol, and the culture was incubated for 14 days on a rotary shaker at the optimal temperature. All volatile substances were added in the amount of 0.5% by volume.

The spectrum of utilized substrates and some biochemical properties of the strain under study were identified using API tests (API 20E, API 20NE; Biomerieux, France) according to the manufacturer's instructions. Growth in the atmosphere of methane, dichloromethane, or $H_2/CO_2/O_2$ was analyzed as described [8].

The ability of the culture to utilize different nitrogen sources was investigated in the K medium, with $(NH_4)_2SO_4$ replaced by the tested substances with an equimolar amount of nitrogen.

Antibiotic sensitivity was determined by the disc diffusion method (Bioanalyse, Turkey).

Production of indole from L-tryptophan was determined with the Salkowski reagent [9]. The calibration curve was plotted using the standard indoleacetic acid solutions.

The phosphate-solubilizing activity was determined in the K medium with insoluble $Ca_3(PO_4)_2$ as the only phosphorus source as described [10].

The ability to synthesize siderophores was detected by the universal chemical method [11] using liquid and agarized K media without $FeCl_3$ with chromeazurol S reagent (CAS) (Sigma, United States). The catechol-type siderophores were tested in the culture liquid of the strain according to Arnow [12].

Electron microscopy. Electron microscopy of the cells was carried out as described [13].

Chemotaxonomic analysis. Ubiquinones were extracted from lyophilized cells, purified by the method of Collins [14], and analyzed in a Finnigan MAT 8430 MS mass spectrometer (Germany).

The fatty acid composition of the cells grown for 48 h on the agar medium with methanol was determined by the known method [15]. The phospholipid composition of the cells was analyzed by two-dimensional thin-layer chromatography [16].

Enzymological analysis was carried out in a cell-free extract as described [17]. The γ-glutamylmethylamide(γ-GMA) lyase and *N*-methylglutamate(*N*-MG) lyase activities were determined by the rate of formaldehyde formation from γ-GMA and *N*-MG, respectively [6]. Enzyme activities were expressed as nanomoles of the substrate transformed or of the product formed in 1 min per mg of protein. Protein was assayed by the Lowry method [18].

DNA isolation and analysis. DNA was isolated using ZR Fungal/Bacterial DNA MiniPrep kit (Zymo Research, United States) according to the manufacturer's instructions.

The G+C content of the DNA was determined by thermal denaturation using a Beckman DU-8B spectrophotometer (United States) at a heating rate of 0.5°C/min and calculated by the following equation: mol G+C = ($T_m \times 2.08$) – 106.4 [19]. The DNA of *Escherichia coli* K-12 was used as a standard. The level of DNA–DNA homology between the Side1^T strain and the type representatives of the genus *Methylopila* was determined by the DNA–DNA reassociation method [20].

The 16S rRNA gene was amplified by PCR using the 27f and 1492r universal prokaryotic primers for 16S rDNA [21].

The fragment (547 bp) of the *mxaF* gene encoding the large subunit of the classical pyrroloquinoline-quinone(PQQ)-dependent methanol dehydrogenase of gram-negative bacteria was amplified using the 1003f and 1561r primers according to the protocol described previously [22].

The fragment (257 bp) of the *mauA* gene encoding the small subunit of methylamine dehydrogenase, which catalyzes the oxidation of methylamine to formaldehyde in halophilic (*Methylophaga* spp.) and nonhalophilic methylobacteria (*Methylobacterium,*

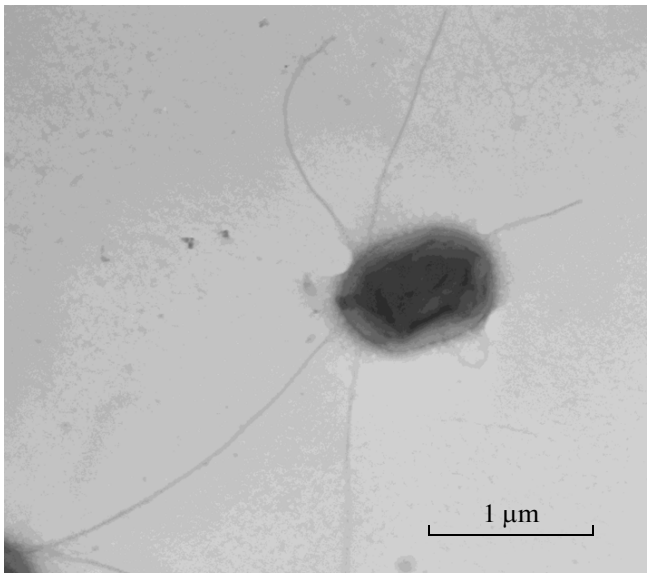

Fig. 1. Cell morphology of the Side1^T strain (negative contrasting).

Paracoccus, Methylophilus, Methylobacillus), was amplified using the f1 and r1 primers [23].

The reaction products were separated by electrophoresis in 1% agarose gel. DNA fragments were isolated and purified from low-melting agarose using the Zymoclean Gel DNA Recovery Kit (Zymo Research, United States) according to the manufacturer's instructions. PCR fragments were sequenced using the CEQ Dye Terminator Cycle Sequencing kit (Beckman Coulter, United States) on a CEQ2000 XL analyzer (Beckman Coulter, United States).

Phylogenetic analysis. Preliminary phylogenetic screening of the similarity between the nucleotide sequences of the 16S rRNA, *mxaF*, and *mauA* genes of the Side1^T strain was performed in the GeneBank [NCBI] database using BLAST software [http://ncbi.nlm.nih.gov]. For more exact phylogenetic identification of the isolate, the nucleotide sequences of the 16S rRNA, *mxaF*, and *mauA* genes were manually aligned with the sequences of taxonomically close reference strains using the CLUSTAL W software package [http://www.genebee.msu.su/ clustal]. The rooted phylogenetic tree was constructed by the neighbor-joining method in TREECON [24]. The evolutionary distance was calculated as a number of substitutions per 100 nucleotides. The statistical reliability of branching was estimated by the "bootstrap analysis" of 100 alternative trees using the respective function of TREECON.

MALDI-TOF/MS analysis. The MALDI spectrum of bacteria was obtained using a MALDI-TOF Autoflex Speed mass spectrometer (Bruker Daltonik GmbH, Germany) according to the procedure described previously [25].

RESULTS AND DISCUSSION

Morphology of the isolate. The Side1^T strain was represented by motile (3–5 flagella) short rods (0.8–1.0 × 1.0–1.5 μm) (Fig. 1) with the gram-negative cell wall type. Capsules and spores were not formed; reproduction was by binary fission. The colonies on the agarized K medium with methanol were pinpoint (1.0–2.0 mm in diameter), opaque, white, glistening, with a convex profile and even edge, smooth surface, and homogenous consistency.

Cultural, physiological, biochemical, and chemotaxonomic properties. The strain under study grew in a liquid K medium with methanol or methylamine hydrochloride without cell aggregation and did not form pigments.

The isolate was strictly aerobic, catalase- and oxidase-positive, and did not need vitamins. Growth occurred on methanol, ethanol, methylamine, trimethylamine, acetate, malate, succinate, fructose, glycerol, and glutamate. It did not grow on methane, dichloromethane, sucrose, D-melibiose, L-rhamnose, L-arabinose, D-glucose, D-sorbitol, D-maltose, D-mannose, *N*-acetylglucosamine, potassium gluconate, capronic and adipic acids, citrate, phenylacetic acid, inositol, amygdalin, dimethylamine, LB (Luria–Bertani) medium, Tryptone soya broth or agar (Sigma), and in the $H_2/CO_2/O_2$ atmosphere. Ammonium, nitrates, methylamine, glutamate, and glutamine were utilized as nitrogen sources. It did not utilize peptone, urea, and serine as nitrogen sources. The API tests (API 20E, 20NE) showed the presence of tryptophan deaminase and urease activities. The isolate was incapable of nitrate reduction. Lysine- and β-glycosidase (esculin hydrolysis), arginine hydrolase, β-galactosidase, ornithine decarboxylase, lysine decarboxylase, and gelatinase activities were absent. Acetoin and indole derivatives were formed (4.8 ± 0.2 μg/mL of the culture liquid at OD_{600} of the culture 1.0) on the medium with nitrate as a nitrogen source, 0.5% methanol and 1% tryptophan. Insoluble phosphates were solubilized: up to 167.3 ± 6.1 μM of phosphorus ions was released in the experiment with $Ca_3(PO_4)_2$ (0.5 g/L). Catechol-type siderophores were synthesized.

Growth occurred at 20–40°C, pH 6.0–9.0. The optimal growth temperature was 29–32°C; the pH optimum was 7.0. The optimal NaCl concentration in the medium was 0.5–2.5%; growth was inhibited by 3% NaCl.

The strain was sensitive to the following antibiotics (mg/mL): oxacillin (5), kanamycin (30), nalidixic acid (30), streptomycin (10), chloramphenicol (30), and tetracycline (30); it was resistant to neomycin (30), novobiocin (30), erythromycin (15), lincomycin (10), penicillin (10), and gentamycin (10).

The predominant fatty acids in the cells were *cis*-11-octadecenoic (C18:1ω7c, 76.44%), 11-methyl octadecenoic (C18:1ω7c11Me, 5.31%), and stearic

Table 1. Activities of the enzymes of primary and intermediate metabolism in cell extracts of strain Side1^T grown on methanol. Standard deviation is ±5%

Enzyme	Cofactor	Activity, nmol/(min mg protein)
Methanol dehydrogenase	PMS*	415
Formaldehyde dehydrogenase	PMS	45
	NAD^+	0
	NAD^++GSH**	4
Formate dehydrogenase	PMS	47
	NAD^+	0
3-Hexulose phosphate synthase	NADH	0
Ribulose-1,5-bisphosphate carboxylase	NADPH	0
Hydroxypyruvate reductase	NADH	1005
	NADPH	322
Serine glyoxylate aminotransferase	NADH	422
	NADPH	115
α-Ketoglutarate dehydrogenase	NADH	20
Isocitrate dehydrogenase	NAD^+	4
	$NADP^+$	305
Isocitrate lyase		0
Pyruvate dehydrogenase	NAD^+	97
Glutamate dehydrogenase	NADH	51
	NADPH	105
Glutamate synthase	NADH	24
	NADPH	33
Glutamate synthetase	ATP, Mn^{2+}	480

* PMS, phenazine methosulfate.

** GSH, reduced glutathione.

(C18:0, 4.46%) acids. Hydroxyl acids (2-OH C19:1, 2-OH C21:1) were also present in minor amounts. The dominant phospholipids were phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, and diphosphatidylglycerol. The dominant ubiquinone was Q_{10}.

Enzymological analysis. The results of enzymological analysis of the cells grown on methanol are listed in Table 1.

The Side1^T strain oxidized methanol to formaldehyde by the classical methanol dehydrogenase stimulated by ammonium ions and showing the maximum activity at pH 9.0. The activities of formaldehyde and formate dehydrogenases with the artificial electron acceptor phenazine methosulfate (PMS) were higher than in the case of NAD-dependent forms of these enzymes. The isolate had high activities of the key enzymes of the serine pathway: L-serine glyoxylate aminotransferase and hydroxypyruvate reductase. The isocitrate lyase activity was absent; consequently, the isocitrate lyase-negative variant of the serine pathway of C_1 metabolism was implemented. The activities of the key enzymes of the ribulose monophosphate and ribulose bisphosphate pathways (hexulose phosphate synthase and ribulose bisphosphate carboxylase) were absent. The activity of the NADP-dependent form of isocitrate dehydrogenase was detected. The presence of α-ketoglutarate dehydrogenase activity indicated the operation of the complete Krebs cycle. Ammonium was assimilated via the glutamate cycle as demonstrated by glutamate synthetase and glutamate synthase activities. At the same time, the glutamate dehydrogenase activity was detected, indicating the possibility of ammonia nitrogen assimilation also by the reductive amination of α-ketoglutarate.

Previously, we have shown that the member of the genus *Methylopila* (*M. musalis* MUSA^T) directly oxidizes methylamine by means of methylamine dehy-

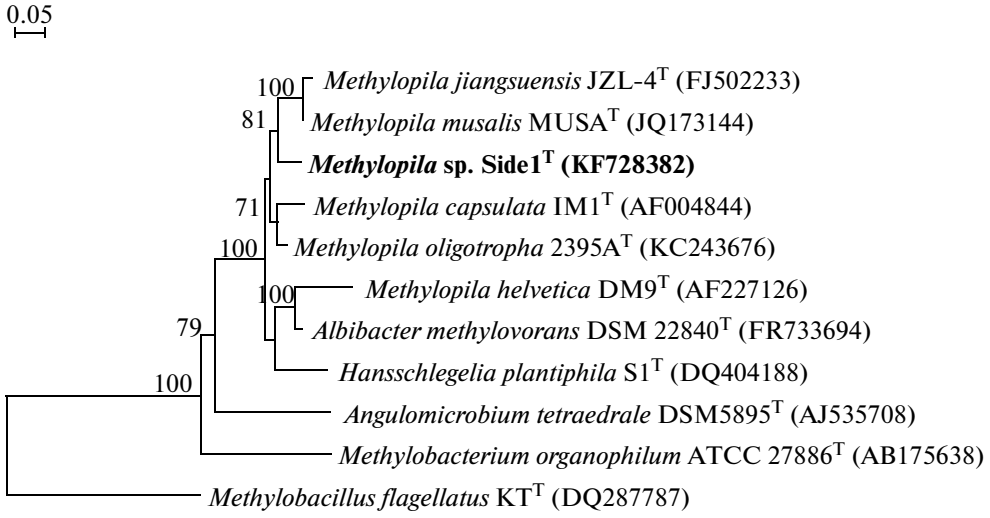

Fig. 2. Phylogenetic position of the Side1^T strain based on the results of comparative analysis of the 16S rRNA gene sequences. The scale corresponds to 5 nucleotide substitutions per every 100 nucleotides (evolutionary distances). The root was determined by inclusion of the sequence of *Methylobacillus flagellates* KT^T (DQ287787) as an outgroup. The numerals show statistical reliability of the branching order determined by bootstrap analysis of 100 alternative trees.

drogenase, as well as via the system of specific enzymes of the *N*-methylglutamate pathway (*N*-methylglutamate lyase/dehydrogenase and γ-glutamylmethylamide lyase) [6]. However, the Side1^T strain oxidized methylamine only by means of methylamine dehydrogenase. *N*-Methylglutamate lyase/dehydrogenase and γ-glutamylmethylamide lyase were not found in the cells grown on methylamine.

Genotypic characteristics. Phylogenetic analysis of the 16S rRNA gene sequence from the Side1^T strain showed its similarity with those of representatives of the genus *Methylopila*: 98.0% with *M. musalis* MUSA^T and *M. oligotropha* 2395A^T and only 97.2–97.3% with other *Methylopila* species (Fig. 2).

According to the data of thermal denaturation of DNA, the G+C content in the strain was 65.4 mol %. The level of DNA–DNA homology of the Side1^T strain with the type representatives of the genus *Methylopila* (*M. musalis* MUSA^T, *M. capsulate* IM1^T, and *M. oligotropha* 2395A^T) was 32, 37, and 34%, respectively.

According to the sequencing data of a fragment of the methanol dehydrogenase gene *mxaF* coding for the large subunit of the enzyme, the Side1^T strain showed the maximum similarity with *M. jiangsuensis* JZL-4^T and *M. helvetica* DM9^T (97.1 % identity of the translated amino acid sequences of mxaF), *M. musalis* MUSA^T (96.0%), *M. capsulate* IM1^T (95.4%), *Hansschlegelia plantiphila* S1^T (92.2%), and *Methylobacterium extorquens* DSM 6343^T (84.2 %) (Fig. 3).

We have also proposed to use the gene of the small methylamine dehydrogenase subunit (*mauA*) for phylogenetic characterization of the genus *Methylopila*. Phylogenetic position of the Side1^T strain based on the comparison of MauA amino acid sequences is shown in Fig. 4. One can see that the Side1^T strain has the high level sequence similarity (98.8%) by the MauA protein with *Methylopila musalis* MUSA^T and some bacteria from other genera: *Methylobacterium chloromethanicum* CM4 (YP_002419416) and *M. extorquens* AM1 (YP_002963808) (97.6%), *Paracoccus denitrificans* SD1 (AEJ28086) and *P. denitrificans* PD1222 (YP_918490) (89.4%), and *Hyphomicrobium sulfonivorans* (ABS45456) (85.7%).

The MALDI-TOF/MS analysis of type strains of the genus *Methylopila* has shown high resolution of this method for the *Methylopila* species, since the strains were well differentiated on the basis of protein profiles. The protein profile of the Side1^T strain was different from those of the members of the genus *Methylopila* (Fig. 5), confirming the new species status of the strain under study.

Thus, the Side1^T strain isolated from the phyllosphere of *Bougainvillea* is a facultative methylotroph utilizing methanol, methylamine, and trimethylamine as the carbon and energy sources via the serine pathway of C_1 metabolism. Based on its pheno- and genotypic characteristics, the strain Side1^T was classified as a novel species of the genus *Methylopila*: *Methylopila turkiensis*. The Side1^T strain differs from the known members of this genus in a number of phenotypic characteristics (Table 2). Obviously, Side1^T is not a random inhabitant of the phyllosphere. This methylotroph uses plant metabolites and, in its turn, synthesizes auxins (indole derivatives) and siderophores, solubilizes insoluble phosphates, and can positively influence plant growth, i.e., it is a phytosymbiont.

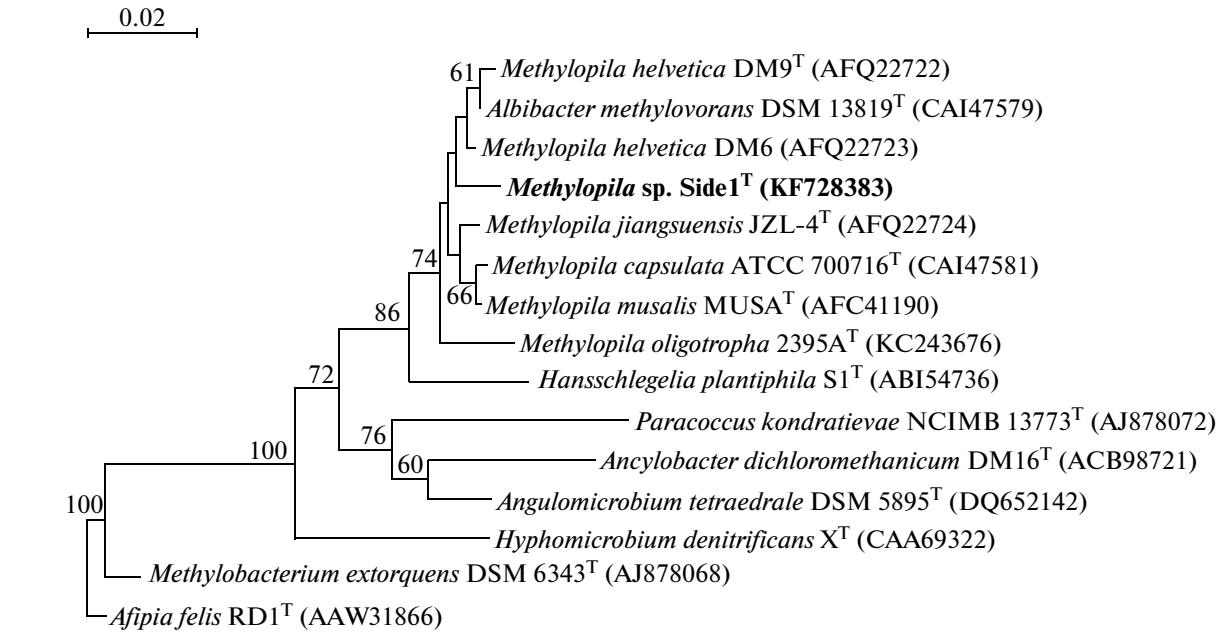

Fig. 3. Phylogenetic position of the Side1T strain based on comparison of amino acid sequences of the MxaF protein. The scale corresponds to 2 nucleotide substitutions per 100 nucleotides (evolutionary distances). The root was determined by inclusion of the sequence of *Afipia felis* RD1T as an outgroup. The numerals show statistical reliability of the branching order determined by the bootstrap analysis of 100 alternative trees.

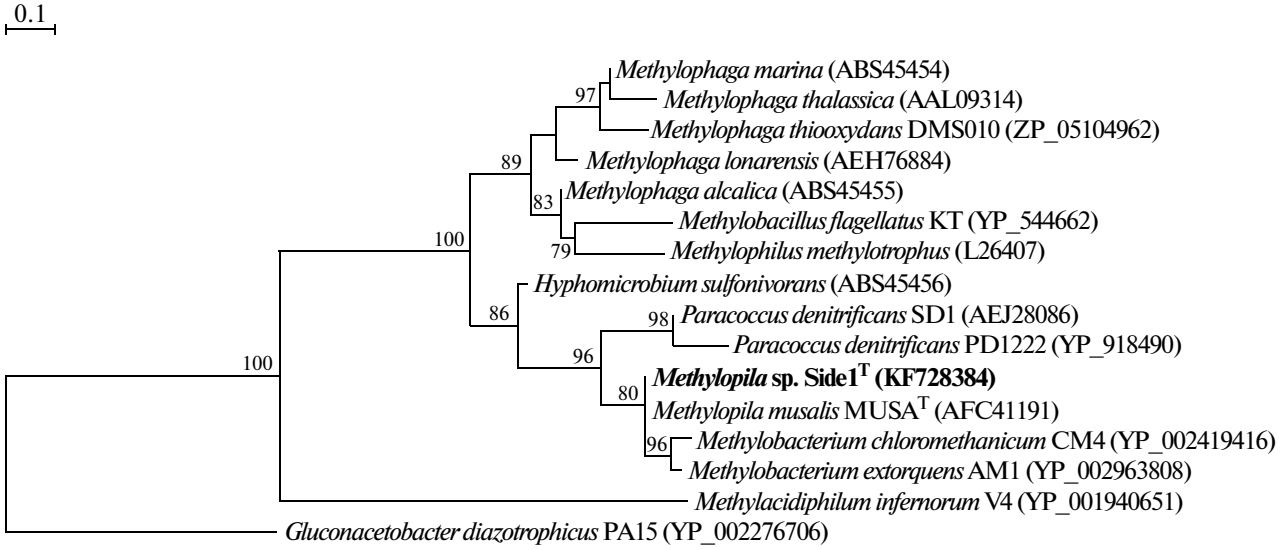

Fig. 4. Phylogenetic position of the Side1 strain based on comparison of amino acid sequences of the MauA protein. The scale corresponds to 10 nucleotide substitutions per every 100 nucleotides (evolutionary distances). The root was determined by inclusion of the sequence of *Gluconacetobacter diazotrophicus* PAl5 (YP_002276706) as an outgroup. The numerals show statistical reliability of the branching order determined by the bootstrap analysis of 100 alternative trees.

Description of Methylopila turkiensis sp. nov.

Methylopila turkiensis sp. nov. (tur. ki. en'sis. N. L. fem. adj.—*turkiensis*—New Latin adjective) was named for the country of the source of isolation, i.e., Turkey.

The cells of strain Side1T are gram-negative, non-spore-forming short rods (0.8–1.0 × 1.0–1.5 μm) reproducing by binary fission. The cells are motile due to the presence of 3–5 flagella. The colonies on the agarized K medium with methanol are pinpoint (1.0–

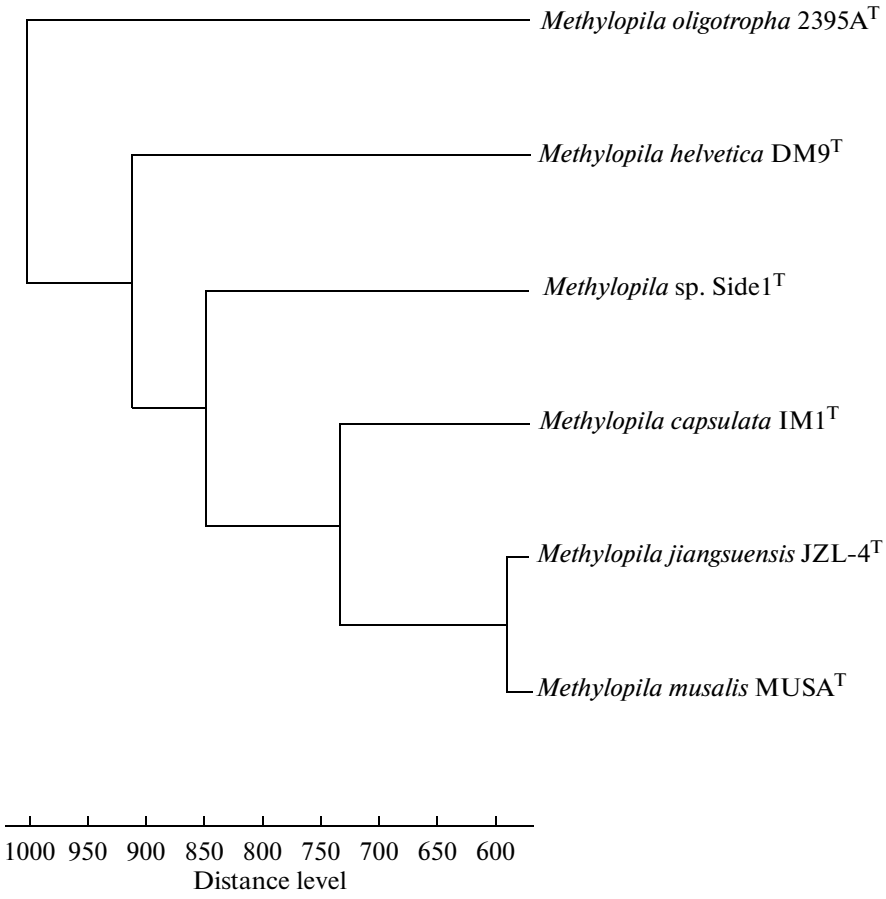

Fig. 5. The dendrogram of methylobacteria of the genus *Methylopila* constructed on the basis of MALDI analysis.

2.0 mm in diameter), opaque, white, glistening, with a convex profile, smooth surface, even edge, and homogeneous structure.

Growth occurs at 20–40°C, pH 6.0–9.0. The growth temperature optimum is 29–32°C; the pH optimum is 7.0. The optimal NaCl concentration in the medium is 0.5–2.5%; growth is inhibited by 3% NaCl.

Utilizes methanol, methylamine, trimethylamine, fructose, glycerol, ethanol, malate, acetate, succinate, and glutamate as the carbon and energy sources. The nitrogen sources are ammonium salts, nitrates, methylamine, and some amino acids. Does not need vitamins and other growth factors. No growth occurs with D-glucose, L-arabinose, D-mannitose, D-mannitol, *N*-acetyl glucosamine, D-maltose, potassium gluconate, capronic and adipic acids, sodium citrate, phenylacetate, inositol, D-sorbitol, L-rhamnose, sucrose, D-melibiose, amygdalin, methane, dichloromethane, and in the $H_2/CO_2/O_2$ atmosphere.

Strictly aerobic; forms catalase, oxidase, and urease. Forms acetoin. Does not hydrolyze gelatin, esculin, cellulose, casein, and starch; does not form hydrogen sulfide. Has a tryptophan deaminase activity. The ability to reduce nitrate and the activities of β-galactosidase, ornithine decarboxylase, and lysine decarboxylase are absent.

Oxidizes methanol and methylamine by means of the relevant dehydrogenases. Implements the isocitrate lyase-negative variant of the serine pathway of C_1 metabolism. Glutamate dehydrogenase and glutamate cycle (glutamate synthase and glutamate synthetase) are involved in ammonium assimilation.

Synthesizes indole derivatives and siderophores; solubilizes insoluble phosphates.

Phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, and diphosphatidylglycerol are predominant in the phospholipid profile of the cells. The predominant fatty acids in the cells are *cis*-11-octadecenoic (C18:1ω7c), 11-methyl-octadecenoic (C18:1ω7c11Me), and stearic (C18:0) acids. The major ubiquinone is Q_{10}. The G+C DNA content is 65.4 mol % (T_m).

The type strain *Methylopila turkiensis* Side1^T (VKM B-2248^T = DSM 27566^T) was isolated from the phyllosphere of bougainvillea (*Bougainvillea* sp. L.); the sample was taken in the town of Side (Turkey).

The 16S rRNA, *mxaF* and *mauA* gene sequences of the Side1^T strain were deposited in the Gen-

Table 2. Differentiating characteristics of members of the genus *Methylopila*

Characteristic	*M. turkiensis* Side1^T	*M. musalis* MUSA^T	*M. jiangsuensis* JZL-4^T	*M. capsulata* IM1^T	*M. helvetica* DM9^T	*M. oligotropha* 2395A^T
Nitrate reduction	–	–	+	–	+	–
Hydrolysis of:						
esculin	–	–	–	+	–	–
gelatin	–	–	–	+	+	–
Assimilation of:						
glycerol	+	–	+	+	+	–
ethanol	+	–	–	+	+	–
D-fructose	+	+	+	+	–	–
L-arabinose	–	–	+	+	–	–
D-sorbitol	–	–	+	+	–	–
maltose	–	–	+	+	–	–
sucrose	–	–	+	+	–	–
succinate	+	–	+	+	+	–
malate	+	–	+	+	–	–
dimethylamine	–	+	–	+	–	+
dichloromethane	–	–	–	–	+	–

Bank/EMBL/DDBL under accession numbers KF728382, KF728383, and KF728384, respectively.

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