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ORIGINAL ARTICLE

Sinomonas mesophila sp. nov., isolated from ancient fort soil

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A moderate growing strain designated as MPKL 26^T was isolated from a soil sample of Bidar Fort, Karnataka, India. The strain MPKL 26^T was Gram positive, bent rod in shape. The optimum pH and temperature for growth was 7.0 and 30 °C, respectively. The 16S ribosomal RNA gene sequence analysis revealed that strain MPKL 26^T was closely related to *S. atrocyanea* DSM 20127^T (98.09%), *S. flava* CW 108^T (98.04%), *S. soli* CW 59^T (97.99%) and *S. notoginsengisoli* SYP-B575^T (97.0%) and showed DNA–DNA hybridization relatedness (46.05 ± 1.2, 33.56 ± 2.55, 32.56 ± 1.7 and 26.79 ± 2.5, respectively, between these strains) less than the threshold value for the delineation of genomic species. The peptidoglycon type was A3α type with glycine, alanine, lysine and glutamic acid as the amino acids. The whole-cell sugars were fructose, ribose, mannose, glucose and galactose. The polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol along with three unknown polar lipids. The fatty acid profile contained $C_{14:0}$, $C_{16:0}$, iso-$C_{14:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and summed feature 4 (17:1 iso I/anteiso B). The predominant respiratory quinine was MK-9(H_2) with MK-10(H_2), MK-8(H_2) and MK-8(H_4) as minor respiratory quinines. The G+C content of the genomic DNA was 68.8 mol%. On the basis of phenotypic, chemotaxonomic and molecular characteristics, the strain MPKL 26^T represents a novel species of the genus *Sinomonas*, for which the name *Sinomonas mesophila* sp. nov. is proposed with MPKL 26^T as the type strain (= NCIM 5552^T = JCM 30094^T).
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INTRODUCTION

The genus *Sinomonas* was first proposed by Zhou *et al.*[1] with the newly isolated strain *S. flava* CW 108^T (the type species of the genus) and *S. atrocyanea* DSM 20127^T (previously classified as *Arthrobacter atrocyaneus*). Soon after the genus published, another two species *Arthrobacter echigonensis* and *Arthrobacter albidus* were reclassified to the genus *Sinomonas* as *S. echigonense* and *S. albida.*[2] At the time writing, one more species of this genus, *S. notoginsengisoli* was proposed by Zhang *et al.*[3] The characteristic features of this genus are: the cells are bent rod in shape and has a high G+C content (66.6–71.8 mol%).[1–4] During the investigation on the biodiversity of microorganisms from soils of Bidar Fort (Karnataka, India), one strain designated as MPLK 26^T was isolated; the 16S ribosomal RNA (rRNA) gene sequence analysis revealed that strain MPKL 26^T was closely related to *S. atrocyanea* DSM 20127^T (98.09%), *S. flava* CW 108^T (98.04%), *Si. soli* CW 59^T (97.99%) and *S. notoginsengisoli* SYP-B575^T (97.0%). The low 16S rRNA gene sequence relatedness (<98.5%) encouraged us to carry out the phenotypic, chemotaxonomic and molecular characterization in order to classify the taxonomic position of the strain MPLK 26^T. On the basis of these results, it was found that the strain MPKL 26^T represents a novel species of the genus *Sinomonas*, for which the name *S. mesophila* sp. nov. is proposed.

MATERIALS AND METHODS

Strain and culture conditions

Strain MPKL 26^T was isolated from the soil sample collected from Bidar Fort (17°55′19″N 77°31′24″E), Karnataka, India, by serial dilution plating method using International Streptomyces project (ISP) 4 medium.[5] The purified strain was maintained on yeast extract–malt extract agar (ISP 2 medium)[5] slants at 4 °C for short-term preservation and as glycerol suspensions (20%, v/v) at − 80 °C for long-term preservation. The reference strains *S. atrocyanea* DSM 20127^T, *S. flava* CW 108^T, *S. soli* CW 59^T and *S. notoginsengisoli* SYP-B575^T were selected for the comparison of phenotypic characterization, DNA–DNA relatedness evaluation and chemotaxonomic determination.

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Phenotypic characteristics

The morphological, physiological and biochemical characters were observed on YDC and TYB or PYES agar media at 30 °C unless mentioned.[6,7] Gram staining was carried out by using the standard Gram reaction. The morphological properties of strain MPKL 26^T were observed with the aid of light microscopy (BH-2; Olympus optical co. Ltd., Tokyo, Japan) and scanning electron microscopy (QUANTA 200; FEI company, Hillsboro, OR, USA). For scanning electron microscopy, cultured cells were harvested by centrifugation, washed and suspended in 20 mM phosphate buffer (pH 7.0). The suspended cells were fixed with 2.5% glutaraldehyde. The cells were dehydrated in an ethanol series (v/v) ranging from 30, 60, 90 and 100%. Cells were dried to a critical drying point. Further, samples were coated with gold and observed under a scanning electron microscope. Growth at various temperature range (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 °C) and pH range 4.0–10.0 (at intervals of 1.0 pH unit) was performed using the buffer system as described by Xu *et al.*[8] The sodium chloride tolerance at various concentrations (0, 0.5, 1, 1.5, 2, 3 and 5.0% w/v) was observed. Catalase activity was determined based on the production of bubbles after the addition of a drop of 3% (v/v) H_2O_2. Oxidase activity was determined based on oxidation of tetramethyl p-phenylenediamine.[9] Cellulose, gelatin, starch; Tweens (20, 40, 60 and 80) hydrolysis; milk coagulation and peptonization were performed as described by Gonzalez *et al.*[10] and other biochemical test was performed by standard methods.[11] The enzymatic activities were determined by the API ZYM stripe (bioMérieux, France) according to the manufacturer's instruction. Utilization of various substrates as sole carbon sources and chemical sensitivity assays was determined by Biolog GN III (Biolog Inc., Hayward, CA, USA) microplates according to the manufacturer's instruction.

Chemotaxonomy

The isomer of amino acids in purified cell wall and whole-cell sugar hydrolysates were determined according to the procedures described by Hasegawa *et al.*,[12] Lechevalier and Lechevalier[13] and Tang *et al.* (a, b).[14,15] Polar lipids were extracted as described by Minnikin *et al.*[16] and identified by two-dimensional TLC.[17] Menaquinones were extracted and analyzed using HPLC.[18,19] Cellular fatty acid analysis was performed by using the Microbial Identification System (Sherlock Version 6.1; MIDI database: TSBA6; Sasser 1990). Biomass for fatty acid analysis was obtained from cell grown on tryptose soy agar (Difco, Sparks, MD, USA) at 30 °C for 4 days.

Molecular analysis

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene of the strain MPKL 26^T was performed by our earlier standard protocol.[20] The sequence obtained was compared with available 16S rRNA gene sequences of cultured species from the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/).[21] Phylogenetic analysis was performed using the software package MEGA version 5.0[22] after multiple alignment of the sequences using CLUSTAL_X program.[23] Distances (using distance options according to Kimura's two-parameter model; Kimura)[24] were calculated and clustering was performed with the neighbor-joining method.[25] To determine the support of each clade, bootstrap analysis was performed with 1000 replications.[26] The validity of the neighbor-joining tree was evaluated with maximum-likelihood tree using MEGA 5.0. (Arizona State University, Phoenix, AZ, USA).[27,28] The G+C content of the genomic DNA was determined by using reversed phase HPLC using *Escherichia coli* DH5α as the reference strain.[29] The DNA–DNA hybridizations with MPKL 26^T and its four reference strains (*S. atrocyanea* DSM 20127^T, *S. flava* CW 108^T, *S. soli* CW 59^T and *S. notoginsengisoli* SYP-B575^T) were carried out by using optical renaturation methods, using eight replications for each hybridization reaction.[30]

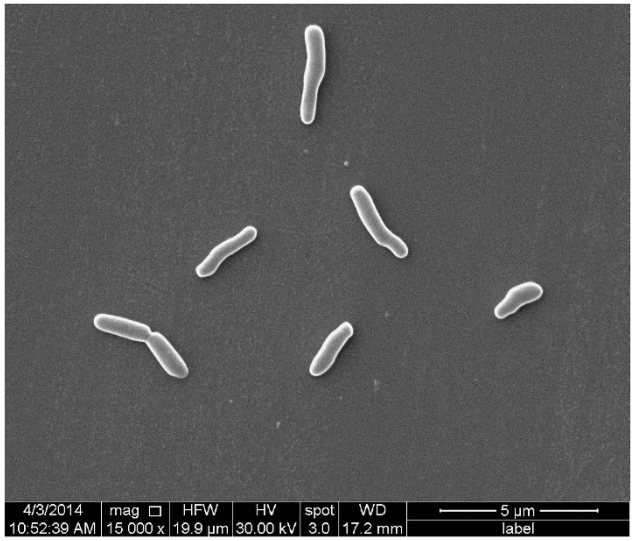

Figure 1 Scanning electron microscope image of strain MPKL 26^T after growth on ISP 2 medium at 30 °C for 2 days.

RESULTS AND DISCUSSION

Phenotypic characteristics

Strain MPKL 26^T was found to be Gram positive, aerobic and non-motile. The scanning electron microscope image (Figure 1) revealed that the cells were bent rod in shape, which is the peculiar character of the genus *Sinomonas*.[1] Temperature for growth ranged from 20 to 40 °C with the optimum growth at 30 °C. The pH for growth ranged from 6 to 8 with the optimum at pH 7. The tolerance to sodium chloride was found to be up to 4% (w/v). The strain MPKL 26^T grew well on YDC, PYES or TYB with no dark blue color on YDC media, this characteristic feature differentiates it from the strain *S. atrocyanea* DSM 20127^T. Catalase and Voges–Proskauer test were positive, but oxidase, H_2S and indole were negative. Milk coagulation was positive, whereas milk peptonization was negative. The strain hydrolyzed Tween 40 weakly, but not for the other Tweens (20, 60 and 80); this feature differentiated the strain from the other reference type strains (DSM 20127^T, CW59^T and CW108^T). The strain MPKL 26^T could utilize dextrin, D-trehalose, D-fructose, 3-methyl glucose, D-glucose-6-PO_4, D-fructose-6-PO_4, D-aspartic acid, L-aspartic acid, L-glutamic acid, L-histidine, D-galacturonic acid, D-glucuronic acid, α-keto-glutaric acid, D-malic acid and L-malic acid, whereas pectin and methyl pyruvate were weakly utilized; these sources of utilization found consistence in reference type strains (DSM 20127^T, CW59^T and CW108^T). In contrast to the above, D-fucose was only utilized by the strain MPKL 26^T. In addition to the above, the production of valine arylamidase and sensitivity to fusidic acid and vancomycin were differential characteristic of the strain MPKL 26^T. A details characteristic features of the strain MPKL 26^T and its type strains were mentioned in Tables 1 and 2.

Chemotaxonomic characteristics

The peptidoglycon type was A3α type with glycine, alanine, lysine and glutamic acid as the amino acids. The strain MPKL 26^T contains fructose, ribose, mannose, glucose and galactose as whole-cell sugars; in comparison with the strain MPKL 26^T, the whole-cell sugar, fructose was devoid in the other type strains (*S. atrocyanea* DSM 20127^T, *S. flava* CW 108^T, *S. soli* CW 59^T and *S. notoginsengisoli* SYP-B575^T). The polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol along with three unknown polar lipids (Supplementary Figure S1). The cellular fatty acid compositions (%) of strain MPKL 26^T and its reference type strains were mentioned in Table 3. The predominant respiratory quinine was MK-9(H_2) with MK-10(H_2), MK-8(H_2) and MK-8(H_4) as minor respiratory quinines.

Phylogenetic analysis and DNA–DNA relatedness

An almost complete 16S rRNA gene sequence (1528 bp) was obtained. The blast result indicated that the strain MPKL 26^T showed high

Table 1 Morphological and physiological characteristics of strain MPKL 26^T and the related type strains of the genus *Sinomonas*

Characteristic	*1*	*2*	*3*	*4*	*5*
Colony color on					
YDC agar	CW	DB	PY	CW	PY
TYB or PYES agar	CW	GW	PY	PY	PY
Citric acid	W	–	+	W	W
Catalase	+	+	W	+	+
Tween 80	–	–	W	+	–
API ZYM					
Esterase (C-4)	+	W	+	+	+
Esterase lipase (C-8)	+	W	+	+	+
Leucine arylamidase	+	+	+	W	+
Valine arylamidase	+	–	–	–	+
Phosphohydrolase	+	+	W	+	+
β-Galactosidase	–	–	W	W	–
β-Glucuronidase	–	–	+	+	+
α-Glucosidase	+	W	+	+	+
β-Glucosidase	+	–	+	+	+
α-Mannosidase	–	–	W	W	–
Utilization of					
Glycerol	+	–	+	–	+
D-glucose	+	+	+	–	+
Sorbitol	+	–	W	–	+
N-acetylglucosamine	W	–	–	+	W
Salicin	+	–	W	–	W
Cellobiose	W	–	W	–	W
Maltose	W	–	W	–	+
Sucrose	W	+	W	+	+
Turanose	+	W	W	+	+
D-maltose	W	W	W	W	+

Abbreviations: CW, creamy white; DB, dark blue; GW, gray white; PY, pale yellow.
Strains: (1) MPKL 26^T; (2) *S. atrocyanea* DSM 20127^T; (3) *S. flava* CW 108^T; (4) *S. soli* CW. 59^T; (5) *S. notoginsengisoli* SYP-B575^T.+, positive; –, negative; W, weakly positive.

Table 2 Chemical and antibiotic susceptibility of strain MPKL 26^T and the related type strains of the genus *Sinomonas*

Chemicals/antibiotics	*1*	*2*	*3*	*4*	*5*
Fusidic acid	S	W	W	W	W
Guanidine hydrochloride	S	S	S	S	S
Lithium chloride	S	W	S	S	S
Sodium butyrate	R	R	R	R	R
Sodium bromated	W	W	W	S	W
Tetrazolium violet	W	R	W	W	W
Tetrazolium blue	S	R	S	S	S
Nalidixic acid	R	R	R	R	R
Niaproof 4	S	S	S	S	S
Troleandomycin	W	W	W	W	W
Lincomycin	W	R	W	W	W
Minocycline	W	W	W	W	W
Rifamycin	S	W	W	W	W
Vancomycin	S	W	W	W	W

Strains: (1) MPKL 26^T; (2) *S. atrocyanea* DSM 20127^T; (3) *S. flava* CW 108^T; (4) *S. soli* CW 59^T; (5) *S. notoginsengisoli* SYP-B575^T. S, sensitive; R, resistant; W, weakly positive.

similarities to *S. atrocyanea* DSM 20127^T (98.09%), *S. flava* CW 108^T (98.04%), *S. soli* CW 59^T (97.99%) and *S. notoginsengisoli* SYP-B575^T (97.0%). The obtained sequence was submitted to GenBank under the accession number (KJ809567). The neighbor-joining tree (Figure 2) showed that the strain MPKL 26^T clustered closely with the members of the genus *Sinomonas*. The cluster further found stable when the tree constructed by using maximum-likelihood method (Supplementary Figure S2). On the basis of phylogenetic analysis, the new isolate MPKL 26^T should be affiliated to the genus *Sinomonas*.

Table 3 Cellular fatty acid compositions (%) of strain MPKL 26^T and its reference type strains: (1) MPKL 26^T, (2) *S. atrocyanea* DSM 20127^T, (3) *S. flava* CW 108^T, (4) *S. soli* CW 59^T and (5) *S. notoginsengisoli* SYP-B575^T

Fatty acids	*1*	*2*	*3*	*4*	*5*
$C_{12:0}$	—	—	1.1	—	—
$C_{14:0}$	0.6	—	0.7	0.5	—
$C_{12:0}$ 3OH	—	—	0.5	—	—
$C_{16:0}$	2.0	1.2	2.6	2.2	1.1
$C_{18:1}$ *w9c*	—	—	0.9	—	—
Iso-$C_{13:0}$	—	—	0.5	—	—
Iso-$C_{14:0}$	0.8	1.6	0.6	2.3	—
Iso-$C_{15:0}$	31.3	21.8	29.0	14.1	23.6
Iso-$C_{16:0}$	4.2	4.8	1.5	11.2	1.3
Iso-$C_{17:0}$	5.1	1.1	1.3	1.4	2.7
Anteiso-$C_{15:0}$	35.9	57.0	43.1	51.3	53.9
Anteiso-$C_{17:0}$	15.4	8.5	6.0	11.6	11.2
Summed feature 3 (16:1 w7c/16:1 w6c)	—	—	4.4	—	—
Summed feature 4 (17:1 iso I/anteiso B)	3.9	2.5	6.1	4.1	4.3

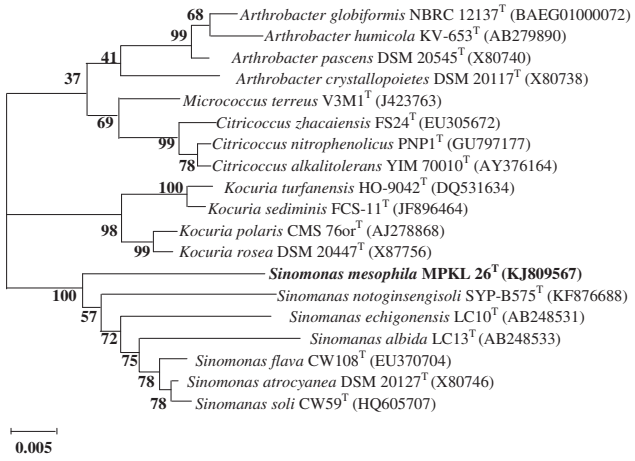

Figure 2 Neighbor-joining phylogenetic tree based on 16S ribosomal RNA gene sequence, showing the position of strain MPKL 26^T. Bootstrap values were shown as percentages of 1000 replicates.

The G+C content of strain MPKL 26^T was determined to be 68.8 mol%, which was within the range of the members of the genus *Sinomonas* (66.6–71.8 mol%).[1–4] DNA–DNA hybridization values between strain MPKL 26^T and its closest phylogenetic neighbors, *S. atrocyanea* DSM 20127^T, *S. flava* CW 108^T, *S. soli* CW 59^T and *S. notoginsengisoli* SYP-B575^T were 46.05 ± 1.2, 33.56 ± 2.55, 32.56 ± 1.7, 26.79 ± 2.5, respectively. The DNA–DNA hybridization values were less than the cutoff point (70%), which was considered to be the threshold value for the delineation of genomic species.[31] On the basis of morphological, biochemical and molecular characters, the strain MPKL 26^T represents a novel species of the genus *Sinomonas*, for which we propose the name *S. mesophila* sp. nov.

Description of *S. mesophila* sp. nov.

S. mesophila (me.so'phi.la. N.L. fem. adj. mesophila; refers to temperature-loving character)

Cells are Gram positive, non-motile, bent rods and aerobic in nature. Colonies are creamy white, circular and convex after 24 h cultivation at 30 °C on TYB, YDC or PYES agar, respectively. Mesophilic type of growth occurs with a temperature range (20–40 °C) and pH range (6–8) with the optimum growth at 30 °C and pH 7. Growth occurs up to 4% (w/v) sodium chloride. Catalase and Voges–Proskauer test are positive, but oxidase, H_2S and indole are negative. Milk coagulation is positive, whereas milk peptonization is negative. Tween 40 is weakly hydrolyzed, whereas starch, cellulose, gelatin and Tweens (20, 60 and 80) are not. Dextrin, D-trehalose, D-turanose, D-raffinose, D-melibiose, D-salicin, α-D-glucose, D-fructose, D-galactose, 3-methyl glucose, L-rhamnose, D-sorbitol, D-mannitol, glycerol, D-glucose-6PO_4, D-fructose-6-PO_4, D-aspartic acid, glycyl-L-proline, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-pyroglutamic acid, L-serine, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, mucic acid, quinic acid, D-saccharic acid, D-lactic acid methyl ester, α-keto-glutaric acid, D-malic acid, L-malic acid, γ-amino-butryric acid β-hydroxy-D, L-butyric acid, acetoacetic acid, propionic acid and acetic acid are utilized, whereas N-acetyl neuraminic acid and bromo-succinic acid are not utilized. Activity for esterase (C-4), esterase lipase (C-8), leucine arylamidase, valine arylamidase, acid phosphatase, phospho-hydrolase, α-glucosidase and β-glucosidase are positive. Sensitive to guanidine hydrochloride, fusidic acid, lithium chloride, tetrazolium blue and vancomycin. The peptidoglycan type is A3α with glycine, alanine, lysine and glutamic acid as the amino acids. The whole-cell sugars are fructose, ribose, mannose, glucose and galactose. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol along with three unknown polar lipids. The fatty acid profile contains $C_{14:0}$, $C_{16:0}$, iso-$C_{14:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and Summed feature 4 (17:1 iso I/anteiso B). The predominant respiratory quinine is MK-9(H_2) with MK-10 (H_2), MK-8(H_2) and MK-8(H_4) as a minor respiratory quinine. The G+C content of strain MPKL 26^T is 68.8 mol%.

The type strain is MPKL 26^T (=NCIM 5552^T= JCM 30094^T), which was isolated from the soil of Bidar fort, Karnataka, India.

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