Lysobacter defluvii sp. nov., isolated from municipal solid waste

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A bacterial isolate obtained from soil from a municipal landfill site in India was characterized using a polyphasic taxonomic approach. The colonies of the isolate were found to be yellow and highly mucoid. Comparative analysis of the 16S rRNA gene sequence showed that this isolate constitutes a distinct phyletic line within the genus *Lysobacter*, displaying >3 % sequence divergence with respect to recognized *Lysobacter* species. The generic assignment was confirmed by chemotaxonomic data, which revealed the presence of a fatty acid profile characteristic of members of the genus *Lysobacter* and consisting of saturated, unsaturated, straight-chain and branched-chain fatty acids as well as iso-$C_{11:0}$ 3-OH as hydroxylated fatty acid, and the presence of an ubiquinone with eight isoprene units (Q-8) as the predominant respiratory quinone. The genotypic and phenotypic data show that strain IMMIB APB-9^T merits classification as representing a novel species of the genus *Lysobacter*, for which the name *Lysobacter defluvii* sp. nov. is proposed. The type strain is IMMIB APB-9^T (=CCUG 53152^T=DSM 18482^T).

The genus *Lysobacter* was first proposed by Christensen & Cook (1978) for gliding organisms with high G+C contents (62–70.1 mol%) and with colonies that were very mucoid and cream, pink or yellow-brown in colour. Members of this genus are strongly proteolytic and characteristically lyse a variety of micro-organisms such as Gram-positive bacteria (including actinomycetes), fungi and green algae, as well as nematodes. Recently, 16S rRNA gene sequence analysis has revealed that members of the genus *Lysobacter* form a distinct phylogenetic line within the *Gammaproteobacteria*, being related to the genera *Xanthomonas*, *Pseudoxanthomonas*, *Stenotrophomonas*, *Thermomonas* and *Xylella* (Bae *et al.*, 2005). In addition, analysis of the quinone system and the cellular fatty acids revealed that *Lysobacter* species contain ubiquinone Q-8 as the major respiratory quinone and have fatty acid profiles with a predominance of iso-branched fatty acids. At the time of writing, the genus *Lysobacter* encompasses eight species with validly published names: *Lysobacter antibioticus*, *Lysobacter brunescens*, *Lysobacter concretionis*, *Lysobacter daejeonensis*, *Lysobacter enzymogenes*, *Lysobacter gummosus*, *Lysobacter koreensis* and *Lysobacter yangpyeongensis*. In this paper we describe a bacterial strain that was isolated from a municipal solid waste dumping site at Udupi, India. On the basis of phylogenetic and phenotypic data, it is proposed that this strain (designated IMMIB APB-9^T) be classified as a novel species of the genus *Lysobacter*.

Strain IMMIB APB-9^T was isolated on nutrient agar from a soil sample taken from a municipal landfill site in Udupi, Karnataka State, India. The isolate was subsequently cultivated on brain-heart infusion (BHI) agar (no. 237100; Becton Dickinson) and tryptone soya agar (CM 131; Oxoid) to determine its morphological characteristics. Pigment production was determined by growing the strains at 37 °C for 7 days, with observations being made at 24 h intervals. The physiological properties of strain IMMIB APB-9^T and of the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMMIB APB-9^T is AM283465.

type strains of recognized *Lysobacter* species were determined using tests to assess the hydrolysis of complex substrates, as described previously (Gordon, 1966, 1967; Gordon & Mihm, 1957). Tween 80 hydrolysis was performed as described by Riegel *et al.* (1994). Fermentation tests were performed using the API Coryne, API 20 Strep and API 20E systems (bioMérieux). Assimilation tests were performed using the API 20NE system (bioMérieux). Enzyme reactions and acid production from carbohydrates were read after 3 and 7 days incubation at 37 °C. Salt tolerance was determined by cultivating the organisms in tryptone soya broth supplemented with NaCl at final concentrations in the range 0.0–12.0 %.

Irrespective of previous chemotaxonomic analyses of *Lysobacter* species, all strains were re-examined to guarantee identical conditions. Chemotaxonomic characteristics of strain IMMIB APB-9^T, as well as those for the type strains of recognized *Lysobacter* species, were determined by cultivating the organisms at 37 °C in shake flasks containing BHI broth for 1 week. After being checked for purity at maximum growth, the organisms were killed with formaldehyde (1 %, v/v), harvested by centrifugation, washed with distilled water and then freeze-dried. Lipids were extracted using acid methanolysis as described by Minnikin *et al.* (1980) and by saponification as described in the MIDI manual. Fatty acid methyl esters obtained after acid methanolysis were purified as described by Yassin (1988). After transesterification, extracts were purified by TLC (article no. 1.05554; Merck) using light petroleum benzene (boiling point, 60–80 °C)/acetone (95 : 5, v/v) as the solvent. The TLC plates were then sprayed with 2′,7′-dichlorofluorescein and air-dried. Thereafter, the fatty acids were detected under UV light (366 nm) as bright yellow bands on a fluorescein background: the non-hydroxylated fatty acids migrated at an R_F value of approximately 0.78 and the hydroxylated ones migrated at an R_F value of approximately 0.13. The fatty acid bands were scraped off, extracted from the silica gel with 5 ml chloroform/methanol (2 : 1, v/v) by vortexing and then centrifuged at 3000 r.p.m. for 5 min. The dye was eliminated by washing the extract with 8 ml 0.5 M ammonium hydroxide solution. The green aqueous layer was discarded by aspiration using a Pasteur pipette and the extract was then washed with water to remove the rest of the dye. The water layer was aspirated using a Pasteur pipette and discarded. Finally, the extract was passed through a column (a cotton-plugged Pasteur pipette) filled to a height of approximately 3 cm with water-free sodium sulfate and then washed with diethyl ether. The eluant was collected in a fresh tube and then evaporated to dryness under a stream of nitrogen gas. The dry extract was dissolved in 50–100 µl methanol and 1–4 µl was used for GC-MS. The fatty acids were separated, identified and quantified by GC-MS using Shimadzu apparatus (QP2010) equipped with a 30.0 m capillary column (HP-1 Fa; Agilent) coated with a thin film of dimethylpolysiloxane as the stationary phase. The respiratory quinones were extracted and purified according to Collins *et al.* (1977). Mass spectral analyses of the quinones were recorded in positive-ion mode on a Q-TOF 2 mass spectrometer (Micromass) equipped with a nanospray source, as described by Yassin & Hupfer (2006). For the compounds under study, the major ions observed with electrospray were protonated pseudo-molecular ions, $[M+Na]^+$. The identity of the ubiquinone was verified by

Table 1. Differential physiological characteristics of strain IMMIB APB-9^T with respect to type strains of recognized *Lysobacter* species

Strains: 1, strain IMMIB APB-9^T (*L. defluvii* sp. nov.); 2, *L. antibioticus* DSM 2044^T; 3, *L. brunescens* DSM 6979^T; 4, *L. concretionis* DSM 16239^T; 5, *L. daejeonensis* DSM 17634^T; 6, *L. enzymogenes* DSM 2043^T; 7, *L. gummosus* DSM 6980^T; 8, *L. yangpyeongensis* DSM 17635^T. All of the strains tested are able to hydrolyse casein and gelatin but not chitin, hypoxanthine, keratin, testosterone, Tween 80 or xanthine. All strains are negative for the assimilation of L-arabinose, D-mannitol, capric acid and phenylacetic acid, and all are negative for acid production from L-arabinose, D-glucose, glycogen, D-lactose, D-maltose, D-mannitol, D-raffinose, D-ribose, D-sorbitol, starch, D-sucrose, D-trehalose and D-xylose. All strains are positive for alkaline phosphatase activity, but negative for arginine dihydrolase, *o*-nitrophenyl-*β*-D-galactopyranosidase, *β*-glucuronidase, leucine arylamidase, pyrrolidonyl arylamidase, L-lysine decarboxylase, L-ornithine decarboxylase, nitrate reductase, pyrazinamidase and urease activities. W, Weakly utilized after incubation for 1 week.

Characteristic	1	2	3	4	5	6	7	8
Hydrolysis of:								
Adenine	−	−	−	−	−	−	+	−
Aesculin	−	+	+	−	+	+	+	+
Elastin	+	−	+	−	−	+	+	−
Guanine	−	+	−	−	−	−	−	+
Hippurate	+	−	+	−	−	−	−	+
Tyrosine	−	+	−	−	+	+	+	−
Assimilation as sources of carbon and energy:								
D-Glucose	−	+	−	−	+	+	+	−
D-Mannose	−	+	−	−	−	+	+	−
N-Acetylglucosamine	−	+	−	−	−	+	+	−
D-Maltose	−	−	−	−	+	+	+	−
Gluconate	−	−	−	W	−	−	−	−
Adipic acid	−	−	−	W	−	−	−	−
Malic acid	−	−	−	−	−	W	−	−
Trisodium citrate	+	−	−	−	−	W	−	−
Enzyme activities:								
β-Galactosidase	−	+	−	−	−	+	+	−
α-Glucosidase	−	−	+	−	+	+	−	+
N-Acetyl-*β*-glucosaminidase	−	−	−	−	−	−	−	+
β-Glucosidase	−	+	+	−	+	+	+	+
α-Galactosidase	−	−	−	−	−	+	+	−
p-Nitrophenyl-*β*-D-galactopyranosidase	−	+	−	−	−	+	+	+
L-Tryptophan deaminase	−	−	−	+	+	+	−	+

observing the diagnostic ion at *m/z* 197, which represents the benzylium ion.

DNA was isolated using an Ultra Clean microbial DNA isolation kit (MO BIO Laboratories) by using the method described in the manufacturer's protocol. The DNA G+C contents were determined by HPLC (Mesbah *et al.*, 1989) using 8 phage as the reference. Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene, and purification of the PCR products were carried out using procedures described previously (Rainey *et al.*, 1996). The purified PCR products were sequenced using a *Taq* DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems) as described in the manufacturer's protocol. An Applied Biosystems 310 DNA Genetic Analyzer was used for the electrophoresis of the sequence reaction products. The 16S rRNA gene sequences of recognized *Lysobacter* species (retrieved from GenBank) were added to the ARB database (Ludwig *et al.*, 2004) and aligned using the respective tool of the ARB package. The resulting alignment was corrected manually and then evolutionary trees were inferred using maximum-parsimony (Fitch, 1971), neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. An evolutionary distance matrix was calculated using the correction of Jukes & Cantor (1969). The topologies of the resulting tree were evaluated in bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method, based on 1000 resamplings.

Strain IMMIB APB-9^T consisted of Gram-negative, non-spore-forming, rod-shaped cells. On BHI agar, nutrient agar and tryptone soya agar, colonies were mucoid and yellow in colour. The micro-organism produced a brown pigment that became more marked in older cultures, and is able to swarm and spread, by gliding, on agar plates incubated for 1 week. The micro-organism grew aerobically and was found to be catalase- and oxidase-positive. It hydrolysed casein, elastin, gelatin and hippurate, but not adenine, chitin, aesculin, guanine, hypoxanthine, keratin, starch, testosterone, tyrosine, Tween 80 or xanthine. The physiological properties of strain IMMIB APB-9^T are given in the species description below. Biochemical characteristics (determined in this study) that can be used to distinguish strain IMMIB APB-9^T from recognized *Lysobacter* species are given in Table 1.

To establish the phylogenetic position of strain IMMIB APB-9^T, its 16S rRNA gene sequence was determined in this study. A tree depicting the phylogenetic relationships of this isolate with respect to members of the genus *Lysobacter* is shown in Fig. 1. The novel isolate forms a distinct subline within the genus *Lysobacter*, branching together with the type strains of *L. concretionis* and *L. daejeonensis*, an association underpinned by all of the tree-making algorithms and by a bootstrap percentage of 99 % in the neighbour-joining analysis. Strain IMMIB APB-9^T displayed the highest sequence similarities with respect to *L. concretionis* (96.0 %) and *L. daejeonensis* (96.1 %). However, sequence divergence values of >3 % with respect to recognized lysobacterial species show unequivocally that isolate IMMIB APB-9^T represents a hitherto unknown species (Stackebrandt & Goebel, 1994).

Chemotaxonomically, strain IMMIB APB-9^T possesses chemical markers that support its assignment to the genus *Lysobacter*. Analysis of the non-hydroxylated fraction of the cellular fatty acids revealed the presence of iso-$C_{14:0}$ (0.71 % of total fatty acids), n-$C_{14:0}$ (0.17 %), iso-$C_{15:1}\omega5c$ (0.14 %), iso-$C_{15:0}$ (40.87 %), n-$C_{15:0}$ (0.83 %), iso-$C_{16:0}$ (19.25 %), n-$C_{16:1}\omega7c$ (0.83 %), n-$C_{16:0}$ (2.92 %), iso-$C_{17:1}\omega9c$ (5.84 %), iso-$C_{17:0}$ (11.14 %), n-$C_{17:0}$ (0.76 %) and n-$C_{18:1}\omega7c$ (0.56 %) as the major non-hydroxylated fatty acid methyl esters. In addition, analysis of the hydroxylated fatty acid fraction revealed the presence of iso-$C_{11:0}$ 3-OH (7.21 %), characterized through the fragment at *m/z* 103. These fatty acids, with some minor differences, were also detected in recognized members of the genus *Lysobacter* (Table 2a and b). Mass spectral analysis of the main isoprenoid components isolated from strain IMMIB APB-9^T showed a strong peak at *m/z* 749.45 attributable to $[M+Na]^+$ in the high-mass region. This corresponds to an ubiquinone with eight isoprene units (Q-8).

It is evident from the data presented that isolate IMMIB APB-9^T exhibits overall cellular morphological and chemotaxonomic characteristics consistent with the genus

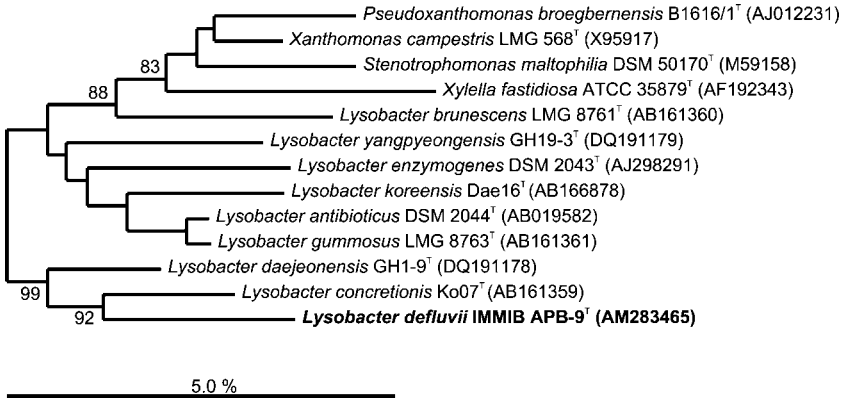

Fig. 1. Neighbour-joining phylogenetic tree, showing the position of strain IMMIB APB-9^T within the radiation of the suborder *Xanthomonadales*. The tree was based on a comparison of 16S rRNA gene sequences that were at least 90 % complete (with regard to the *Escherichia coli* sequence). Numbers at nodes are bootstrap percentages, based on 1000 resamplings. Bar, 5.0 % sequence divergence.

Table 2. Non-hydroxylated cellular fatty acids (expressed as percentages of total fatty acids) of members of the genus *Lysobacter* as determined in this study using GC-MS analysis (a) and the MIDI system (b)

Strains: 1, strain IMMIB APB-9^T (*L. defluvii* sp. nov.); 2, *L. antibioticus* DSM 2044^T; 3, *L. brunescens* DSM 6979^T; 4, *L. concretionis* DSM 16239^T; 5, *L. daejeonensis* DSM 17634^T; 6, *L. enzymogenes* DSM 2043^T; 7, *L. gummosus* DSM 6980^T; 8, *L. yangpyeongensis* DSM 17635^T. −, Not detected.

(a)

Peak no.	*m/z*	Fatty acid	1	2	3	4	5	6	7	8
1	214	iso-$C_{11:0}$	1.78	−	11.65	2.36	6.33	5.95	1.74	6.29
2	228	iso-$C_{13:0}$	−	−	0.32	0.65	0.52	0.65	0.4	0.5
3	242	iso-$C_{14:0}$	0.71	0.30	1.41	0.32	1.69	0.27	−	0.34
4	242	n-$C_{14:0}$	0.17	−	0.82	0.49	0.25	1.27	0.45	0.65
5	254	iso-$C_{15:1}\omega 6c$*	−	−	−	−	−	0.39	−	−
6	254	iso-$C_{15:1}\omega 5c$*	0.14	−	2.47	1.12	1.42	0.75	1.10	1.06
7	256	iso-$C_{15:0}$	40.87	33.91	22.07	15.70	16.47	18.07	26.07	24.0
8	256	anteiso-$C_{15:0}$	−	0.35	−	0.43	0.58	0.36	0.31	0.62
9	256	n-$C_{15:0}$	0.83	−	1.84	0.89	1.23	−	0.82	0.26
10	268	iso-$C_{16:1}\omega 7c$	−	−	−	−	0.65	−	−	0.92
11	270	iso-$C_{16:0}$	19.25	2.03	6.37	7.46	10.90	1.55	0.60	4.57
12	268	n-$C_{16:1}\omega 11c$	−	0.38	−	−	−	−	0.55	0.46
13	268	n-$C_{16:1}\omega 7c$	0.83	2.07	5.47	1.41	−	23.53	4.63	−
14	270	n-$C_{16:0}$	2.92	5.38	4.46	4.01	2.78	10.07	6.61	1.09
15	282	iso-$C_{17:1}\omega 9c$	5.84	20.19	29.09	39.06	44.40	18.11	26.36	26.66
16	282	iso-$C_{17:1}\omega 8c$	−	0.71	0.97	2.88	−	−	1.22	0.99
17	284	iso-$C_{17:0}$	11.14	34.01	10.18	18.21	13.67	12.10	27.14	32.52
18	284	anteiso-$C_{17:0}$	−	−	−	0.07	0.10	0.12	−	−
19	282	$C_{17:0}$ cyclo†	3.20	−	−	1.42	−	0.63	−	−
20	284	n-$C_{17:0}$	0.76	−	0.21	0.19	−	−	−	0.35
21	296	n-$C_{18:1}\omega 9c$	−	−	−	−	−	1.49	0.17	−
22	296	n-$C_{18:1}\omega 7c$	0.56	0.34	0.33	2.89	−	2.57	0.75	0.37
23	298	iso-$C_{18:0}$	−	−	−	−	−	−	−	0.13
24	298	n-$C_{18:0}$	−	0.21	0.25	0.29	−	1.76	0.28	0.39

Lysobacter, but appears not to conform to any recognized species. Phylogenetic analysis based on 16S rRNA gene sequencing confirmed this provisional assignment and clearly demonstrated that isolate IMMIB APB-9^T represents an unknown subline within the genus *Lysobacter*. Biochemically, strain IMMIB APB-9^T can be easily differentiated from the *Lysobacter* species that are most closely related phylogenetically and biochemically (Table 1). On the basis of both phenotypic and phylogenetic evidence, the isolate represents a novel species within the genus *Lysobacter*, for which the name *Lysobacter defluvii* sp. nov. is proposed.

Description of *Lysobacter defluvii* sp. nov.

Lysobacter defluvii [de.flu′vi.i. L. gen. neut. n. *defluvii* from outflow (sewage)].

Cells are non-motile, non-spore-forming rods (approximately 1–2 μm in length). Gram-negative and oxidase- and catalase-positive. Colonies are mucoid and yellow in colour. Produces a brown pigment that becomes more marked in older cultures. Growth occurs at temperatures in the range 22–37 °C. Salt concentrations in the range 0.0–6.0 % (w/v) NaCl are tolerated. Growth occurs on tryptone soya agar and nutrient agar but the micro-organism grows preferentially on BHI agar. Has the ability to swarm and spread on the surface of agar plates by means of gliding. Shows the salient chemotaxonomic characteristics of members of the genus *Lysobacter*. Fatty acids mainly comprise saturated and unsaturated fatty acids with straight and branched chains of the iso/anteiso type. The main hydroxylated fatty acid is iso-$C_{11:0}$ 3-OH. Hydrolyses casein, elastin, gelatin and hippurate, but not adenine, chitin, aesculin, guanine, hypoxanthine, keratin, starch, testosterone, tyrosine, Tween 80 or xanthine. Does not produce acid from L-arabinose, starch, amygdalin, D-glucose, glycogen, inositol, inulin, D-lactose, D-maltose, D-mannitol, D-melibiose, D-raffinose, D-rhamnose, D-ribose, D-sorbitol, D-sucrose, D-trehalose or xylose. Shows alkaline phosphatase activity, but is negative for arginine dihydrolase, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, leucine aminopeptidase, lysine decarboxylase, ornithine

(b)

Retention time	Fatty acid	1	2	3	4	5	6	7	8
3.559	iso-$C_{11:0}$	2.26	–	5.64	1.44	2.69	1.82	0.78	5.19
4.395	iso-$C_{12:0}$	0.37	–	–	–	0.09	–	–	–
5.467	iso-$C_{13:0}$	–	–	0.31	0.48	0.34	0.44	0.32	0.41
6.763	iso-$C_{14:0}$	1.27	–	1.04	0.24	0.93	0.20	–	0.40
7.287	n-$C_{14:0}$	0.53	0.25	0.62	0.34	0.17	0.81	0.29	–
7.896	iso-$C_{15:1}$ AT 5‡	–	0.42	–	–	–	–	0.59	1.77
7.931	iso-$C_{15:1}$ F‡	0.94	–	1.69	0.70	0.94	–	–	–
8.030	iso-$C_{15:1}$ H‡	–	–	0.15	0.13	–	0.48	–	–
8.260	iso-$C_{15:0}$	44.17	54.77	56.14	58.54	51.34	60.0	70.38	39.68
8.392	anteiso-$C_{15:0}$	1.63	0.70	1.34	0.56	0.38	0.39	0.21	0.63
8.613	n-$C_{15:1}\omega 6c$	–	–	0.18	0.13	–	0.08	–	–
8.835	n-$C_{15:0}$	1.17	–	0.90	0.53	0.58	0.12	0.40	0.29
9.603	iso-$C_{16:1}\omega 7c$	–	–	–	–	0.15	–	–	–
9.883	iso-$C_{16:0}$	19.42	1.65	3.82	3.87	11.51	0.89	0.29	3.88
10.107	n-$C_{16:1}\omega 11c$	–	0.37	–	–	–	–	0.29	0.20
10.203	n-$C_{16:1}\omega 7c$	0.94	1.75	3.10	0.92	0.44	11.19	1.99	–
10.506	n-$C_{16:0}$	4.11	4.05	2.61	2.12	1.23	5.0	2.78	1.01
11.233	iso-$C_{17:1}\omega 9c$	6.49	13.07	16.15	19.12	16.47	8.68	10.49	20.80
11.329	iso-$C_{17:1}\omega 8c$	–	–	0.38	1.17	–	0.36	0.32	–
11.600	iso-$C_{17:0}$	16.64	21.82	5.57	8.54	12.42	5.68	10.27	24.06
11.921	anteiso-$C_{17:0}$	0.60	–	–	0.08	0.11	–	–	–
11.890	n-$C_{17:0}\omega 8c$	–	–	–	–	–	–	0.11	–
12.049	n-$C_{17:0}$ cyclo	3.61	–	–	0.69	–	0.20	–	–
12.245	n-$C_{17:0}$	1.25	–	–	0.13	0.09	–	–	0.27
13.603	n-$C_{18:1}\omega 9c$	–	–	–	–	–	0.78	–	–
13.697	n-$C_{18:1}\omega 7c$	–	0.70	–	–	–	1.29	0.34	–
14.007	n-$C_{18:0}$	–	0.45	0.13	0.20	–	0.92	0.15	0.42

*Presumptive position of the double bond.

†Corresponds to methyl 9,10-methylene-hexadecanoate.

‡iso-$C_{15:1}$ AT5/F/H should correspond to either iso-$C_{15:1}\omega 6c$ and/or iso-$C_{15:1}\omega 5c$. The double bond position is presumptive.

decarboxylase, tryptophan deaminase, pyrazinamidase, pyrrolidonyl arylamidase, nitrate reductase and urease activities. Negative for acetoin, indole and H_2S production. Assimilates trisodium citrate but not D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid or phenylacetic acid. The DNA G+C content of the type strain is 67.1 mol%.

The type strain, IMMIB APB-9^T (=CCUG 53152^T=DSM 18482^T), was isolated from a soil sample taken from a municipal landfill site in Udupi, Karnataka State, India.

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