Acinetobacter indicus sp. nov., isolated from a hexachlorocyclohexane dump site

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The taxonomic position of a Gram-negative, non-motile, oxidase negative and catalase positive strain, A648^T, isolated from a hexachlorocyclohexane (HCH) dump site located in Lucknow, India, was ascertained by using a polyphasic approach. A comparative analysis of a partial sequence of the *rpoB* gene and the 16S rRNA gene sequence revealed that strain A648^T belonged to the genus *Acinetobacter*. DNA–DNA relatedness values between strain A648^T and other closely related members (16S rRNA gene sequence similarity greater than 97 %), namely *Acinetobacter radioresistens* DSM 6976^T, *A. venetianus* ATCC 31012^T, *A. baumannii* LMG 1041^T, *A. parvus* LMG 21765^T, *A. junii* LMG 998^T and *A. soli* JCM 15062^T, were found to be less than 8 %. The major cellular fatty acids of strain A648^T were 18 : 1 ω 9c (19.6 %), summed feature 3 (15.9 %), 16 : 0 (10.6 %) and 12 : 0 (6.4 %). The DNA G + C content was 40.4 mol%. The polar lipid profile of strain A648^T indicated the presence of diphosphatidylglycerol, phosphatidylethanolamine, followed by phosphatidylglycerol and phosphatidylcholine. The predominant polyamine of strain A648^T was 1,3-diaminopropane and moderate amounts of putrescine, spermidine and spermine were also detected. The respiratory quinone consisted of ubiquinone with nine isoprene units (Q-9). On the basis of DNA–DNA hybridization, phenotypic characteristics and chemotaxonomic and phylogenetic comparisons with other members of the genus *Acinetobacter*, strain A648^T is found to be a novel species of the genus *Acinetobacter*, for which the name *Acinetobacter indicus* sp. nov. is proposed. The type strain is A648^T (=DSM 25388^T=CCM 7832^T).

The genus *Acinetobacter* was established in 1954 by Brisou & Prevot (1954). Species belonging to this genus have been largely reported from water, soil and even from human skin samples. At the time of writing, this genus included 32 genomic species (Bouvet & Grimont, 1986; Bouvet & Jeanjean, 1989; Tjernberg & Ursing, 1989; Gerner-Smidt & Tjernberg, 1993; Vaneechoutte *et al.*, 1999; Nemec *et al.*, 2001; Carr *et al.*, 2003; Nemec *et al.*, 2003), of which 26 have validly published names (www.bacterio.cict.fr/a/acinetobacter.html). Members of the genus *Acinetobacter* are non-motile, strictly aerobic, oxidase negative, catalase positive and Gram-negative coccobacilli. Other characteristics include their ability to utilize a diverse range of compounds as sources of carbon and to grow on minimal salt medium.

We are studying the microbial diversity at a hexachlorocyclohexane (HCH) dump site that was created as a result of the disposal of α - and β -HCH (HCH waste left out after

the purification of lindane) on barren land located at Ummari village, Lucknow, India (Jit *et al.*, 2011). Several bacteria have been isolated from this dump site. Over the past 5 years, we have been characterizing bacterial strains from this site to augment our efforts to develop bioremediation technology. So far we have characterized 15 novel species from this HCH dump site and these studies indicate that over the past few years the microbial community at the dump site has been drastically altered as a result of the presence of HCH waste, giving rise to bacterial strains that either degrade or tolerate very high levels of α - and β -HCH isomers. Both these HCH degraders and non-degraders seem to play an important role in depleting the HCH isomer levels at these sites (Lal *et al.*, 2010).

In the present investigation, a bacterial strain, A648^T, was isolated from soil samples collected from this HCH dump site. This strain was isolated by plating a serially diluted soil sample on Luria–Bertani (LB) plates. For this, 1 g soil was inoculated in 9 ml minimal salt medium and incubated for 4 days, from which 100 μ l was plated on LB agar containing: 10 g tryptone l⁻¹, 5 g yeast extract l⁻¹, 5 g NaCl l⁻¹, 1 g glucose l⁻¹ and 15 g agar l⁻¹. After 24 h incubation at 28 °C, a yellow-coloured colony appeared that was picked and purified by repeated streaking on LB agar. The taxonomic position of strain A648^T was determined by

Abbreviations: HCH, hexachlorocyclohexane; ML, maximum likelihood; NJ, neighbour-joining; Q-9, ubiquinone.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and partial *rpoB* gene sequences of strain A648^T (=DSM 25388^T=CCM 7832^T) are HM047743 and JF772169, respectively.

Four supplementary figures and two supplementary tables are available with the online version of this paper.

using a polyphasic approach (Kumar *et al.*, 2008; Jit *et al.*, 2008; Singh & Lal, 2009). On the basis of phylogenetic, genotypic and phenotypic characteristics, strain A648^T was found to represent a novel species of the genus *Acinetobacter*.

The 16S rRNA gene sequence of strain A648^T was amplified using the 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1542R (5'-AAGGAGGTGATCCAGCCGCA-3') universal primer set by colony PCR (Kumar *et al.*, 2008). The PCR product was purified using a Gel Extraction kit (Nucleospin Extract II, MACHEREY-NAGEL), according to the manufacturer's instructions. The eluted DNA fragment was then directly sequenced by the dideoxy chain-termination method using the 3100 Avant Genetic Analyzer (Applied Biosystems). The 16S rRNA gene sequence obtained was manually checked and aligned using SEQUENCING ANALYSIS 5.11 and CLONE MANAGER 5. A continuous stretch of 1393 bp of the 16S rRNA gene sequence was obtained and used to search for similarity using the sequence match tool of Ribosomal Database Project II (http://rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp), the BLAST program of the National Centre for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>) and the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). A preliminary sequence comparison with the 16S rRNA gene sequences deposited in the GenBank database indicated that strain A648^T belonged to the genus *Acinetobacter*. The sequence similarity was calculated as the nucleotide differences between the 16S rRNA gene of A648^T and those of the other members of the genus. The search revealed that the nearest neighbours of strain A648^T are *Acinetobacter radioresistens* DSM 6976^T (97.6%), *A. venetianus* ATCC 31012^T (97.5%), *A. baumannii* LMG 1041^T (97.4%), *A. parvus* LMG 21765^T (97.4%), *A. junii* LMG 998^T (97.3%) and *A. soli* JCM 15062^T (97.0%). For the construction of the phylogenetic tree, almost full-length 16S rRNA gene sequences of all the recognized species and some other strains assigned to the genus *Acinetobacter* were retrieved from the GenBank database. These sequences were aligned using the CLUSTAL_X program (Thompson *et al.*, 1997), and the 16S rRNA gene sequence of *Psychrobacter immobilis* ATCC 43116^T, which showed 89% sequence similarity with A648^T, was used as the out-group. To elucidate the phylogenetic relationship between the novel isolate and the other species of the genus *Acinetobacter*, the neighbour-joining (NJ) method of Saitou & Nei (1987) (Fig. 1) and maximum-likelihood (ML) (Takahashi & Nei, 2000) (Fig. S1, available in IJSEM Online) methods were used in the PHYLIP package (Felsenstein, 1993). The phylogenetic tree was analysed by using the TreeView program (Page, 1996). The resultant tree topology was inferred using bootstrap analysis (Felsenstein, 1981) based on 1000 resamplings. The evolutionary distance matrix was generated using the distance model of Jukes & Cantor (1969). The 16S rRNA gene sequence analysis showed the affiliation of strain A648^T to the genus *Acinetobacter*. This result was

supported by the tree constructed using the ML method, where strain A648^T clustered among members of the genus *Acinetobacter*. Thus, the phylogenetic tree also confirmed the observation that strain A648^T belongs to the genus *Acinetobacter*.

The delineation of a novel species within the genus *Acinetobacter* is difficult (Nowak & Kur, 1996) and hence, in addition to the 16S rRNA gene analysis, *rpoB* gene sequence analysis has been demonstrated to be useful (La Scola *et al.*, 2006). To confirm the relatedness of strain A648^T to the genus *Acinetobacter*, and its separation from all recognized members of this genus, comparative sequence analysis of the RNA polymerase β -subunit (*rpoB*) gene was performed according to the method of La Scola *et al.* (2006) and Nemec *et al.* (2009), using two sets of primers to amplify two variable regions of the *rpoB* gene. Zone 1 spans nucleotide positions 2916–3267 [primers Ac696F (5'-TAYCGYAAAGAYTTGAAAGAAG-3') and Ac1093R (5'-CMACACCYTTGTTMCCRTGA-3')], and zone 2 spans nucleotide positions 3263–3773 [primers Ac1055F (5'-GTGATAARATGGCBGGTCGT-3') and Ac1598R (5'-CG-BGCRTGCATYTTGTCRT-3')]. Sequencing analyses were carried out with a BigDye Terminator cycle sequencing kit (Applied Biosystems) and an ABI Prism 3100 Genetic Analyzer. Partial sequences for the *rpoB* gene of strain A648^T, the type strains of recognized species of the genus and other strains assigned to this taxon were used for the construction of the phylogenetic trees. These sequences were aligned using the coding reading frame with CLUSTAL_X (Thompson *et al.*, 1997). The trees were constructed as described earlier. The Jukes–Cantor algorithm was used to calculate the evolutionary distances (Jukes & Cantor, 1969). The calculations were carried out for concatenated zones 1 and 2 using nucleotide positions 2917–3267 for zone 1 and positions 3322–3723 for zone 2. The position numbers correspond to those of the *rpoB*-encoding sequence of *A. baumannii* (La Scola *et al.*, 2006). The phylogram for concatenated *rpoB* zones 1 and 2 is shown in Figs 2 and S2. The interspecies similarity values of strain A648^T with other members of the genus ranged from 86.6 to 81.4%, which is supported by the previous findings of Nemec *et al.* (2010). The similarity values based on the amino acid analysis varied between 95.1 and 88.5%. The resulting tree, using both the NJ and ML methods, as well as the amino acid sequence analysis, depicted that strain A648^T forms a distinct lineage within the genus *Acinetobacter*. The conclusion drawn from the phylogenetic tree of the 16S rRNA gene was supported by the comparative analysis of the *rpoB* gene, that is, that strain A648^T is a novel species of the genus *Acinetobacter*.

For DNA–DNA hybridization studies, the genomic DNA of six type strains that showed 16S rRNA gene sequence similarity greater than 97% was isolated and purified. The DNA–DNA hybridization was carried out by the membrane filter method, as explained by Kumar *et al.* (2008). The results of DNA–DNA hybridization were expressed as the percentage of DNA relatedness (values $\pm$ SDs are presented in Table S1). Each value was the mean of four

Fig. 2. Phylogenetic tree based on two concatenated variable zones of the *rpoB* gene sequences of strain A648^T and representative members of the genus *Acinetobacter*. The tree was constructed using the NJ method. Bootstrap values >70% are indicated (based on 1000 resamplings). GenBank accession numbers are given in parentheses. *Pseudomonas aeruginosa* PAO1 (GenBank accession no. NC_002516) was used as the out-group. Bar, 0.01 nucleotide substitutions per site.

reported to possess a characteristic polyamine distribution pattern with 1,3-diaminopropane (DAP) as a major component and very little putrescine, spermidine or spermine (Busse & Auling, 1988; Yamamoto *et al.*, 1991). Since DAP has been reported to be the characteristic main polyamine compound produced by many members of the genus *Acinetobacter*, it has also been used as a biomarker for the identification of the genus (Auling *et al.*, 1991). On analysis, the major polyamine of strain A648^T was found to be DAP and moderate amounts of putrescine, spermidine and spermine were also detected, demonstrating that strain A648^T belongs to the genus *Acinetobacter*.

Polar lipid analysis of strain A648^T was performed by two dimensional TLC, as described by Gupta *et al.* (2009). The total lipid profile was detected by spraying aqueous primuline solution, which consisted of 100 µl 1% primuline solution and 100 µl water that was added to 10 ml acetone, with the solution being mixed well. This solution was sprayed on TLC plates and spots were visualized under UV light. The polar lipid analysis of strain A648^T revealed the presence of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylcholine

(Fig. S3). This profile was similar to those of the recently described strains of the genus *Acinetobacter* (Lee & Lee, 2010; Aluyi *et al.*, 1992).

Quinones were extracted according to the method explained by Dadhwal *et al.* (2009) and analysed by reverse-phase TLC, according to Collins & Jones (1980). Ubiquinone Q-9 was detected as the respiratory quinone in strain A648^T. The presence of this quinone system supported the fact that strain A648^T belongs to the genus *Acinetobacter* (Kim *et al.*, 2008; Nishimura *et al.*, 1988; Collins & Jones, 1981). All these chemotaxonomic data suggest that strain A648^T represents a novel species of the genus *Acinetobacter*.

The DNA G + C content of strain A648^T, as determined by using the thermal denaturation method of Gonzalez & Saiz-Jimenez (2002), was found to be 40.4 mol%, which was within the range as reported for the genus by Vaz-Moreira *et al.* (2011).

The colour, size and shape of cells of strain A648^T were observed on LB agar plates incubated at 28 °C for 24 h. Gram staining was performed using HiMedia Gram

Table 1. Cellular fatty acid profile of strain A648^T and the type strains of closely related members of the genus *Acinetobacter* (16S rRNA gene sequence similarity greater than 97 %)

1, Strain A648^T; 2, *A. radioresistens* DSM 6976^T; 3, *A. venetianus* ATCC 31012^T; 4, *A. baumannii* LMG 1041^T; 5, *A. parvus* LMG 21765^T; 6, *A. junii* LMG 998^T; 7, *A. soli* JCM 15062^T. The data were generated for all strains by streaking them on LB agar at 28 °C for 48 h. Values are percentages of total fatty acids. —, Not detected.

Fatty acid	1	2	3	4	5	6	7
10:0	0.8	0.5	2.5	—	—	2.5	0.4
12:0	6.4	11.2	5.1	9.7	5.9	3.8	10.5
13:0	1.2	—	—	0.4	—	0.2	—
14:0	1.7	1.3	0.7	0.7	1.7	1.3	0.6
16:0	10.6	15.6	18.2	17.6	16.8	16.3	20.2
17:0	1.5	1.2	2.4	2.4	0.7	1.7	0.5
18:0	4.6	1.5	0.7	0.9	1.0	2.3	0.9
12:0 2-OH	2.2	0.9	7.6	4.6	3.9	7.6	3.9
12:0 3-OH	4.2	6.5	9.2	5.9	5.6	8.9	6.5
17:1ω8c	1.8	2.2	2.9	3.0	1.7	1.0	0.3
18:1ω9c	19.6	25.8	25.2	34.9	38.2	28.1	36.6
17:0 anteiso	1.2	—	—	0.2	—	0.2	0.2
Summed feature*							
2	4.4	2.4	0.4	6.6	—	0.4	4.0
3	15.9	12.3	22.4	11.3	23.8	23.5	12.9
5	—	15.8	—	—	—	—	—
8	1.4	1.5	1.0	—	—	—	—

*Summed features are groups of two or three fatty acids that cannot be separated using the MIDI system. Summed feature 2 consists of 16:1 iso1 and/or 14:0 3-OH, summed feature 3 consists of 16:1ω7c and/or 16:1ω6c, summed feature 5 consists of 18:0 ante and/or 18:2ω6,9c and summed feature 8 consists of 18:1ω6c and/or 18:1ω7c.

staining kit (HiMedia). The morphology of colonies, streaked on LB agar plates and incubated for 24 h, was examined under a light microscope (model 2000; Motic). Growth at different temperatures, pH and NaCl concentrations was determined as described by Arden-Jones *et al.* (1979). In order to determine the growth at different temperatures, strain A648^T was streaked on LB agar plates and incubated at 4, 10, 28, 37, 42, 44 and 55 °C for 4–5 days. Growth was studied at different pH values (range of pH 4–12) and salt concentrations (1–10 %, w/v, NaCl) in LB broth at 28 °C. Strain A648^T was cultured to test its growth on tryptic soy agar (TSA) and nutrient agar (NA) at 28 °C. The motility of strain A648^T cells was checked on motility agar medium (Farmer, 1999). Cell morphology and the presence or absence of flagella was examined by taking a loopful of culture grown until stationary phase (at 28 °C for 24 h), suspending it in a fixative and washing with a phosphate buffer, and then observing the sample under a transmission electron microscope (TEM; Morgagni 269D; Fei). The cells were then negatively

stained with 0.5 % uranyl acetate and the grids were observed under a TEM (All India Institute of Medical Sciences) (Fig. S4).

Haemolysis was tested on Columbia agar plates supplemented with 5 % sheep blood (HiMedia) within 48 h of incubation. Acid production from carbohydrates and other substrates was tested using the basal mineral medium of Cruze *et al.* (1979), supplemented with filter-sterilized carbon source solutions at final concentrations of 0.2 % (w/v, carbohydrates) and 0.1 % (w/v, other substrates), as described by Nishimura *et al.* (1988). The basal medium consisted of the following (l⁻¹): 10 g KH₂PO₄, 5 g Na₂HPO₄, 2 g (NH₄)₂SO₄, 0.2 g MgSO₄·7H₂O, 0.001 g CaCl₂·2H₂O and 0.001 g FeSO₄·7H₂O (pH 7.0). The tubes were incubated at 28 °C and growth on carbon sources was evaluated after 2, 4, 6 and 10 days.

Hydrolysis of aesculin, casein, gelatin, DNA, and Tween 20 and 80 was tested as described by Arden-Jones *et al.* (1979). β-Galactosidase activity was observed using HiMedia ONPG discs. The catalase test was carried out using 3 % (v/v) hydrogen peroxide ascertained by McCarthy & Cross (1984). The nitrate reduction test was performed as described by Smibert & Krieg (1994). Other physiological tests were carried out as described by Collins *et al.* (1989).

Strain A648^T is a Gram-negative, non-motile, aerobic bacterium. Strain A648^T appeared light yellow, convex and smooth with circular colonies after 24 h of incubation at 28 °C on LB agar. Strain A648^T was negative in tests for oxidase activity and positive for catalase. Several morphological and phenotypic characteristics, as observed in this study, differentiated strain A648^T from related members of the genus *Acinetobacter* (Tables 2 and S2). Selected phenotypic characteristics of strain A648^T determined in this study were also compared with the 26 validly named species (Table 2). The study revealed that strain A648^T is a non-haemolytic *Acinetobacter* strain, which has the ability to utilize benzoate, ethanol, DL-lactate, phenylacetate, but not mannose, D-fructose, raffinose, cellobiose, xylose, mannitol, meso-inositol, sucrose, sorbose, citraconate, adipate, putrescine, L-aspartate, L-histidine, L-arginine, L-ornithine, L-lysine, β-alanine or D-malate as a single carbon source (Tables 2 and S2). Thus, based on the phylogenetic, biochemical and genotypic analyses, we conclude that strain A648^T represents a novel species of the genus *Acinetobacter* for which the name *Acinetobacter indicus* sp. nov. is proposed.

Description of *Acinetobacter indicus* sp. nov.

Acinetobacter indicus (in'di.cus. L. masc. adj. *indicus* Indian, of or belonging to India).

Gram-negative, non-motile, non-spore-forming, aerobic bacterium. Colonies are light yellow in colour, small, entire, smooth, circular, convex and opaque. Optimum growth is observed within 24 h on LB medium at 28 °C. Grows well on LB, NA and TSA. The colony size obtained

Table 2. Distinctive characteristics of the different species of members of the genus *Acinetobacter*

Taxa: 1, Strain A648^T (data from this study); 2, *A. radioresistens* (Nemec *et al.*, 2011); 3, *A. venetianus* (Vanechoutte *et al.*, 2009); 4, *A. baumannii* (Nemec *et al.*, 2011); 5, *A. parvus* (Nemec *et al.*, 2011); 6, *A. junii* (Nemec *et al.*, 2011); 7, *A. soli* (Kim *et al.*, 2008); 8, *A. bereziniae* (Nemec *et al.*, 2011); 9, *A. guillouiae* (Nemec *et al.*, 2011); 10, *A. lwoffii* (Nemec *et al.*, 2011); 11, *A. beijerinckii* (Nemec *et al.*, 2009); 12, *A. haemolyticus* (Bouvet & Grimont, 1986); 13, *A. johnsonii* (Nemec *et al.*, 2011); 14, *A. baylyi* (Nemec *et al.*, 2011); 15, *A. gerneri* (Nemec *et al.*, 2011); 16, *A. tandoii* (Nemec *et al.*, 2011); 17, *A. ursingii* (Nemec *et al.*, 2011); 18, *A. bouvetii* (Nemec *et al.*, 2011); 19, *A. calcoaceticus* (Nemec *et al.*, 2011); 20, *A. gyllenbergii* (Nemec *et al.*, 2009); 21, *A. tjernbergiae* (Carr *et al.*, 2003); 22, *A. towneri* (Nemec *et al.*, 2011); 23, *A. schindleri* (Nemec *et al.*, 2011); 24, *A. rudis* (Vaz-Moreira *et al.*, 2011); 25, *A. nosocomialis* (Nemec *et al.*, 2011); 26, *A. pittii* (Nemec *et al.*, 2011); 27, *A. brisouii* (Anandham *et al.*, 2010). +, All strains positive; −, all strains negative; v+, 85–99% of strains positive; v, 16–84% of strains positive; v−, 1–15% of strains positive; NA, not available; D, doubtful or weak reaction.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
No. of isolates	1	6	5	25	10	4	1	16	17	8	15	23	8	5	1	1	15	1	11	9	1	2	14	2	20	20	1
Growth at																											
44 °C	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	v+	v−	−
41 °C	+	+	−	+	−	v	+	−	−	−	−	−	−	v	−	−	−	−	v−	−	−	NA	v+	−	+	+	−
37 °C	+	+	+	+	+	+	+	+	v	+	+	+	v	+	+	+	v+	D	v+	v+	−	+	+	+	+	+	+
Haemolytic activity	−	−	+	−	−	v	−	−	−	−	+	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	NA
Acidification of D-glucose	−	−	−	+	−	−	+	v+	−	v−	−	v	−	+	+	−	−	−	v+	−	−	−	−	−	+	v+	−
Utilization of:																											
Adipate	−	+	v	v+	−	−	+	v	+	v+	−	NA	−	+	+	−	+	−	+	+	−	−	v	−	v+	+	−
β-Alanine	−	−	−	+	−	−	NA	+	v+	−	−	−	−	−	+	−	−	−	v+	+	−	−	−	NA	v+	v+	+
4-Aminobutyrate	D	+	+	+	−	v	NA	+	v+	v+	+	+	v	+	+	+	−	−	+	v−	−	−	−	NA	+	+	−
L-Arabinose	D	−	−	v	−	−	+	−	−	−	−	NA	−	−	−	−	−	−	v	−	NA	−	−	−	+	v+	−
L-Arginine	−	+	+	+	−	+	NA	−	−	−	−	+	v	+	−	+	−	−	+	+	+	−	−	−	+	+	NA
L-Aspartate	−	−	−	+	−	v	+	+	+	−	+	v	v	+	+	+	v+	−	+	−	v	−	−	−	+	+	NA
Benzoate	+	+	+	v	−	+	NA	+	v+	v+	−	NA	+	+	+	+	v	+	+	+	NA	+	v+	+	v+	v+	NA
2,3-Butanediol	D	+	−	+	−	−	NA	+	+	−	−	−	v	+	+	+	+	−	+	−	−	v	v	+	v+	v+	NA
Citraconate	−	−	−	v	−	−	NA	−	−	−	−	NA	−	−	−	−	−	−	−	−	NA	−	−	NA	−	−	NA
Citrate (Simmons)	−	−	+	+	−	v	+	+	+	v−	+	v+	v	+	+	+	+	+	v+	+	−	−	v	+	+	+	−
Ethanol	+	+	+	v+	+	v	NA	+	+	+	+	v+	+	+	+	+	+	+	v+	v	NA	+	v+	+	+	+	NA
D-Glucose	−	−	−	−	−	−	+	−	−	−	−	NA	−	+	−	−	−	−	−	−	NA	−	−	−	−	−	−
L-Histidine	−	−	+	v+	−	v	NA	v+	v+	−	+	v+	−	−	−	+	−	+	+	+	+	−	−	+	+	+	−
DL-Lactate	+	+	−	+	−	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	NA	+	+	NA	+	+	+
D-Malate	−	−	+	v+	−	v	+	v+	+	v−	+	v+	−	D	−	+	v+	−	D	+	NA	v	v+	NA	+	v+	NA
L-Ornithine	−	−	−	v	v	−	NA	−	−	−	−	−	v−	−	−	+	−	−	+	v	NA	−	−	NA	v+	v+	NA
Phenylacetate	+	+	−	v	−	−	+	v	v	v+	−	−	−	−	+	+	−	−	+	+	−	−	−	+	v+	v	+
Putrescine	−	v	−	v+	−	−	NA	−	−	−	−	NA	−	−	−	+	−	−	+	−	−	−	−	−	v+	+	NA
D-Ribose	D	−	NA	v	−	−	+	−	−	−	−	NA	−	−	−	−	−	−	v	−	NA	−	−	−	v	v	−

is 0.5 mm in diameter, after 24 h of incubation at 28 °C. Growth is observed in 0–5 % (w/v) NaCl, over pH 7–9 and at a temperature range of 22–42 °C. The optimal growth is observed at 28 °C, pH 7.0 and 1 % (w/v) NaCl. Tween 20 and 80 are hydrolysed, but not aesculin, casein, gelatin or DNA. The H₂S production test is negative. Not flagellated. Cells are coccobacilli in shape, with a cell size of 1.0 µm as analysed by electron microscopy. The nitrate reduction test is positive. Utilizes ethanol and acetate as sole sources of carbon in basal medium and negative result for the haemolysis of sheep blood. Assimilation of adipate, β-alanine, L-aspartate, L-ornithine, L-arginine, L-lysine and L-histidine is not observed, but the assimilation of benzoate and DL-lactate is observed. The major fatty acids are 18:1ω9c and summed feature 3, comprising (16:1ω7c and/or 16:1ω6c) 16:0 and 12:0. The major polyamine is 1,3-diaminopropane. The respiratory quinone detected is ubiquinone Q-9. The predominant polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylcholine.

The type strain, A648^T (=DSM 25388^T=CCM 7832^T), was isolated from a HCH dump site at Ummari Village, Lucknow, India. The DNA G+C content is 40.4 mol%.

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References

- Aluyi, H. S., Boote, V., Drucker, D. B., Wilson, J. M. & Ling, Y. H. (1992). Analysis of polar lipids from some representative enterobacteria, *Plesiomonas* and *Acinetobacter* by fast atom bombardment-mass spectrometry. *J Appl Bacteriol* **73**, 426–432.
- Anandham, R., Weon, H.-Y., Kim, S.-J., Kim, Y.-S., Kim, B.-Y. & Kwon, S.-W. (2010). *Acinetobacter brisouii* sp. nov., isolated from a wetland in Korea. *J Microbiol* **48**, 36–39.
- Arden-Jones, M. P., McCarthy, A. J. & Cross, T. (1979). Taxonomic and serologic studies on *Micropolyspora faeni* and *Micropolyspora* strains from soil bearing the specific epithet *rectivirgula*. *J Gen Microbiol* **115**, 343–354.
- Auling, G., Pilz, F., Busse, H. J., Karrasch, S., Streichan, M. & Schön, G. (1991). Analysis of the polyphosphate-accumulating microflora in phosphorus-eliminating, anaerobic-aerobic activated sludge systems by using diaminopropane as a biomarker for rapid estimation of *Acinetobacter* spp. *Appl Environ Microbiol* **57**, 3585–3592.
- Bouvet, P. J. M. & Grimont, P. A. D. (1986). Taxonomy of the genus *Acinetobacter* with the recognition of *Acinetobacter baumannii* sp. nov., *Acinetobacter haemolyticus* sp. nov., *Acinetobacter johnsonii* sp. nov. and *Acinetobacter junii* sp. nov. and emended descriptions of *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii*. *Int J Syst Bacteriol* **36**, 228–240.
- Bouvet, P. J. M. & Jeanjean, S. (1989). Delineation of new proteolytic genomic species in the genus *Acinetobacter*. *Res Microbiol* **140**, 291–299.
- Brisou, J. & Prevot, A. R. (1954). Etudes de systématique bactérienne. X. Révision des espèces réunies dans le genre *Achromobacter*. *Ann Inst Pasteur (Paris)* **86**, 722–728.
- Busse, J. & Auling, G. (1988). Polyamine pattern as a chemotaxonomic marker within the *Proteobacteria*. *Syst Appl Microbiol* **11**, 1–8.
- Carr, E. L., Kämpfer, P., Patel, B. K. C., Gürtler, V. & Seviour, R. J. (2003). Seven novel species of *Acinetobacter* isolated from activated sludge. *Int J Syst Evol Microbiol* **53**, 953–963.
- Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.
- Collins, M. D. & Jones, D. (1980). Lipids in the classification and identification of coryneform bacteria containing peptidoglycan based on 2,4-diamino butyric acid (DAB). *J Appl Bacteriol* **48**, 459–470.
- Collins, M. D. & Jones, D. (1981). Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiol Rev* **45**, 316–354.
- Collins, C. H., Lyne, P. M. & Grange, J. M. (1989). *Microbiological Methods*, 6th edn. London: Butterworth.
- Cruze, J. A., Singer, J. T. & Finnerty, W. R. (1979). Conditions for quantitative transformation in *Acinetobacter calcoaceticus*. *Curr Microbiol* **3**, 129–132.
- Dadhwal, M., Jit, S., Kumari, H. & Lal, R. (2009). *Sphingobium chinhatense* sp. nov., a hexachlorocyclohexane (HCH)-degrading bacterium isolated from an HCH dumpsite. *Int J Syst Evol Microbiol* **59**, 3140–3144.
- Farmer, J. J., III (1999). *Enterobacteriaceae*: introduction and identification. In *Manual of Clinical Microbiology*, 7th edn, pp. 448–452. Edited by P. R. Murray, E. J. Baron, M. A. Tenover & R. H. Tenover. Washington, DC: American Society for Microbiology.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Gerner-Smidt, P. & Tjernberg, I. (1993). *Acinetobacter* in Denmark: II. Molecular studies of the *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex. *APMIS* **101**, 826–832.
- Gonzalez, J. M. & Saiz-Jimenez, C. (2002). A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* **4**, 770–773.
- Gupta, S. K., Lal, D. & Lal, R. (2009). *Novosphingobium panipatense* sp. nov. and *Novosphingobium mathurensense* sp. nov., from oil-contaminated soil. *Int J Syst Evol Microbiol* **59**, 156–161.
- Jit, S., Dadhwal, M., Prakash, O. & Lal, R. (2008). *Flavobacterium lindanitolerans* sp. nov., isolated from hexachlorocyclohexane-contaminated soil. *Int J Syst Evol Microbiol* **58**, 1665–1669.
- Jit, S., Dadhwal, M., Kumari, H., Jindal, S., Kaur, J., Lata, P., Niharika, N., Lal, D., Garg, N. & other authors (2011). Evaluation of hexachlorocyclohexane contamination from the last lindane production plant operating in India. *Environ Sci Pollut Res Int* **18**, 586–597.
- Jukes, T. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

- Kämpfer, P. (1993). Grouping of *Acinetobacter* genomic species by cellular fatty acid composition. *Med Microbiol Lett* **2**, 394–400.
- Kim, D., Baik, K. S., Kim, M. S., Park, S. C., Kim, S. S., Rhee, M. S., Kwak, Y. S. & Seong, C. N. (2008). *Acinetobacter soli* sp. nov., isolated from forest soil. *J Microbiol* **46**, 396–401.
- Kumar, M., Verma, M. & Lal, R. (2008). *Devosia chinhatensis* sp. nov., isolated from a hexachlorocyclohexane (HCH) dump site in India. *Int J Syst Evol Microbiol* **58**, 861–865.
- Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358–361.
- La Scola, B., Gundi, V. A., Khamis, A. & Raoult, D. (2006). Sequencing of the *rpoB* gene and flanking spacers for molecular identification of *Acinetobacter* species. *J Clin Microbiol* **44**, 827–832.
- Lal, R., Pandey, G., Sharma, P., Kumari, K., Malhotra, S., Pandey, R., Raina, V., Kohler, H. P.-E., Holliger, C. & other authors (2010). Biochemistry of microbial degradation of hexachlorocyclohexane and prospects for bioremediation. *Microbiol Mol Biol Rev* **74**, 58–80.
- Lee, H. J. & Lee, S. S. (2010). *Acinetobacter kyonggiensis* sp. nov., a β -glucosidase-producing bacterium, isolated from sewage treatment plant. *J Microbiol* **48**, 754–759.
- McCarthy, A. J. & Cross, T. (1984). A taxonomic study of *Thermomonospora* and other monosporic actinomycetes. *J Gen Microbiol* **130**, 5–25.
- Miller, L. T. (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584–586.
- Nemec, A., De Baere, T., Tjernberg, I., Vaneechoutte, M., van der Reijden, T. J. K. & Dijkshoorn, L. (2001). *Acinetobacter ursingii* sp. nov. and *Acinetobacter schindleri* sp. nov., isolated from human clinical specimens. *Int J Syst Evol Microbiol* **51**, 1891–1899.
- Nemec, A., Dijkshoorn, L., Cleenwerck, I., De Baere, T., Janssens, D., Van Der Reijden, T. J. K., Jezek, P. & Vaneechoutte, M. (2003). *Acinetobacter parvus* sp. nov., a small-colony-forming species isolated from human clinical specimens. *Int J Syst Evol Microbiol* **53**, 1563–1567.
- Nemec, A., Musilek, M., Maixnerová, M., De Baere, T., van der Reijden, T. J. K., Vaneechoutte, M. & Dijkshoorn, L. (2009). *Acinetobacter beijerinckii* sp. nov. and *Acinetobacter gyllenbergii* sp. nov., haemolytic organisms isolated from humans. *Int J Syst Evol Microbiol* **59**, 118–124.
- Nemec, A., Musilek, M., Šedo, O., De Baere, T., Maixnerová, M., van der Reijden, T. J. K., Zdráhal, Z., Vaneechoutte, M. & Dijkshoorn, L. (2010). *Acinetobacter bereziniae* sp. nov. and *Acinetobacter guillouiae* sp. nov., to accommodate *Acinetobacter* genomic species 10 and 11, respectively. *Int J Syst Evol Microbiol* **60**, 896–903.
- Nemec, A., Krizova, L., Maixnerova, M., van der Reijden, T. J., Deschaght, P., Passet, V., Vaneechoutte, M., Brisse, S. & Dijkshoorn, L. (2011). Genotypic and phenotypic characterization of the *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex with the proposal of *Acinetobacter pittii* sp. nov. (formerly *Acinetobacter* genomic species 3) and *Acinetobacter nosocomialis* sp. nov. (formerly *Acinetobacter* genomic species 13TU). *Res Microbiol* **162**, 393–404.
- Nishimura, Y., Ino, T. & Iizuka, H. (1988). *Acinetobacter radioresistens* sp. nov. isolated from cotton and soil. *Int J Syst Evol Microbiol* **38**, 209–211.
- Nowak, A. & Kur, J. (1996). Differentiation of seventeen genospecies of *Acinetobacter* by multiplex polymerase chain reaction and restriction fragment length polymorphism analysis. *Mol Cell Probes* **10**, 405–411.
- Page, R. D. M. (1996). TreeView: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* **12**, 357–358.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Singh, A. & Lal, R. (2009). *Sphingobium ummariense* sp. nov., a hexachlorocyclohexane (HCH)-degrading bacterium, isolated from HCH-contaminated soil. *Int J Syst Evol Microbiol* **59**, 162–166.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, D.C.: American Society for Microbiology.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Takahashi, K. & Nei, M. (2000). Efficiencies of fast algorithms of phylogenetic inference under the criteria of maximum parsimony, minimum evolution, and maximum likelihood when a large number of sequences are used. *Mol Biol Evol* **17**, 1251–1258.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Tjernberg, I. & Ursing, J. (1989). Clinical strains of *Acinetobacter* classified by DNA-DNA hybridization. *APMIS* **97**, 595–605.
- Vaneechoutte, M., Tjernberg, I., Baldi, F., Pepi, M., Fani, R., Sullivan, E. R., van der Toorn, J. & Dijkshoorn, L. (1999). Oil-degrading *Acinetobacter* strain RAG-1 and strains described as '*Acinetobacter venetianus* sp. nov.' belong to the same genomic species. *Res Microbiol* **150**, 69–73.
- Vaneechoutte, M., Nemec, A., Musilek, M., van der Reijden, T. J., van den Barselaar, M., Tjernberg, I., Calame, W., Fani, R., De Baere, T. & Dijkshoorn, L. (2009). Description of *Acinetobacter venetianus* ex Di Cello *et al.* 1997 sp. nov. *Int J Syst Evol Microbiol* **59**, 1376–1381.
- Vaz-Moreira, I., Novo, A., Hantsis-Zacharov, E., Lopes, A. R., Gomila, M., Nunes, O. C., Manaia, C. M. & Halpern, M. (2011). *Acinetobacter rudis* sp. nov., isolated from raw milk and raw wastewater. *Int J Syst Evol Microbiol* **61**, 2837–2843.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Yamamoto, S., Tsuzaki, Y., Kishi, R. & Nakao, H. (1991). Occurrence of L-2,4-diaminobutyrate decarboxylase activity in *Acinetobacter*. *Chem Pharm Bull (Tokyo)* **39**, 2451–2453.