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 : 1ω9*c* (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 µl 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

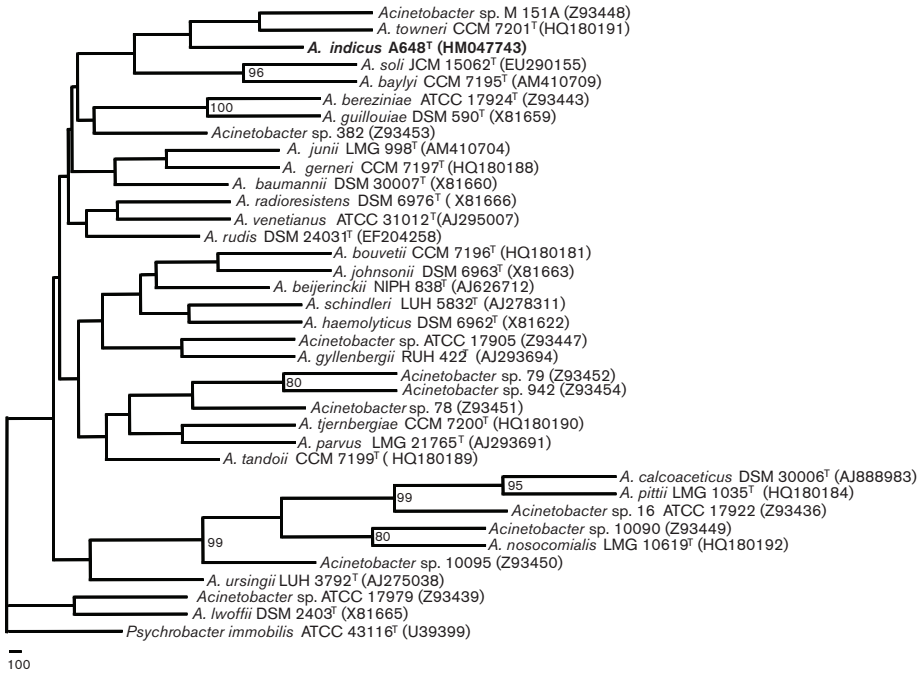

Fig. 1. Phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationship of strain A648^T with the other members of the genus *Acinetobacter*. *P. immobilis* ATCC 43116^T (GenBank accession no. U39399) was used as the out-group. The tree was constructed using the NJ method. The numbers at nodes represent bootstrap values (based on a resampling of 1000). Bootstrap values >70 % are indicated. The GenBank accession numbers for the 16S rRNA gene sequences of each reference species are listed in parentheses. Bar, 0.01 nucleotide substitutions per site.

replicates. The DNA–DNA relatedness values between strain A648^T and its nearest neighbours were found to be less than 8 %, (7.3 % with *A. radioresistens* DSM 6976^T, 6.9 % with *A. venetianus* ATCC 31012^T, 6.0 % with *A. baumannii* LMG 1041^T, 5.5 % with *A. parvus* LMG 21765^T, 3.8 % with *A. junii* LMG 998^T and 2.5 % with *A. soli* JCM 15062^T). As the DNA–DNA hybridization values were below the threshold value (70 %) that has been recommended for the delineation of a species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994), strain A648^T represents a novel species of the genus *Acinetobacter*.

Fatty acid methyl ester (FAME) analysis of strain A648^T and six recognized species of the genus that are the nearest neighbours of strain A648^T, namely *A. 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, was carried out. All strains were grown on LB agar medium at 28 °C for 24 h, then sent to Disha Institute of Biotechnology, Ahmadabad, India, for fatty acid analysis. At the Disha Institute, the cultures were revived on trypticase soy broth agar (TSBA) and grown for 24 h, after which cultures from the third quadrant were picked and then subjected to fatty acid analysis. FAMEs were analysed from 2–4 loops of inoculum scraped from a Petri dish and subjected to saponification, methylation and extraction using the methods of Miller (1982) and Kuykendall *et al.* (1988). The FAME mixtures were separated using the Sherlock Microbial Identification System (MIDI) and identification of fatty acids was made by using the Aerobe (TSBA, 6.0 version) database. The predominant fatty acids of strain A648^T were 18 : 1ω9*c* (19.6 %), summed feature 3 (16 : 1ω7*c* and/or 16 : 1ω6*c*, 15.9 %), 16 : 0 (10.6 %) and 12 : 0 (6.4 %). Fatty acid analysis also indicated that strain A648^T contained summed feature 2, comprising 14 : 0 3-OH and/or 16 : 1 iso 1 (4.4 %), 12 : 0 3-OH (4.2 %) and 12 : 0 2-OH (2.2 %) (Table 1). The presence of 12 : 0, 12 : 0 3-OH, 16 : 0, 18 : 1ω9*c* and summed feature 3 fatty acids is a feature shown by most of the members of the genus *Acinetobacter* (Kim *et al.*, 2008). A significant difference was observed in terms of the percentage of each type of cellular fatty acid between that found in strain A648^T and those in the other recognized species of this genus (Kämpfer, 1993). This profile thus confirms that A648^T represents a novel species of the genus *Acinetobacter*.

For the polyamine analysis, polar lipid profile and detection of quinones, the culture was inoculated in LB broth and incubated at 28 °C for 24 h and was then lyophilized to obtain the dry cell mass. Polyamines were then extracted as described by Busse & Auling (1988) and analysed by one dimensional TLC (Silica gel 60 F254, 20 × 20 cm; Merck). Some members of the genus *Acinetobacter* have been

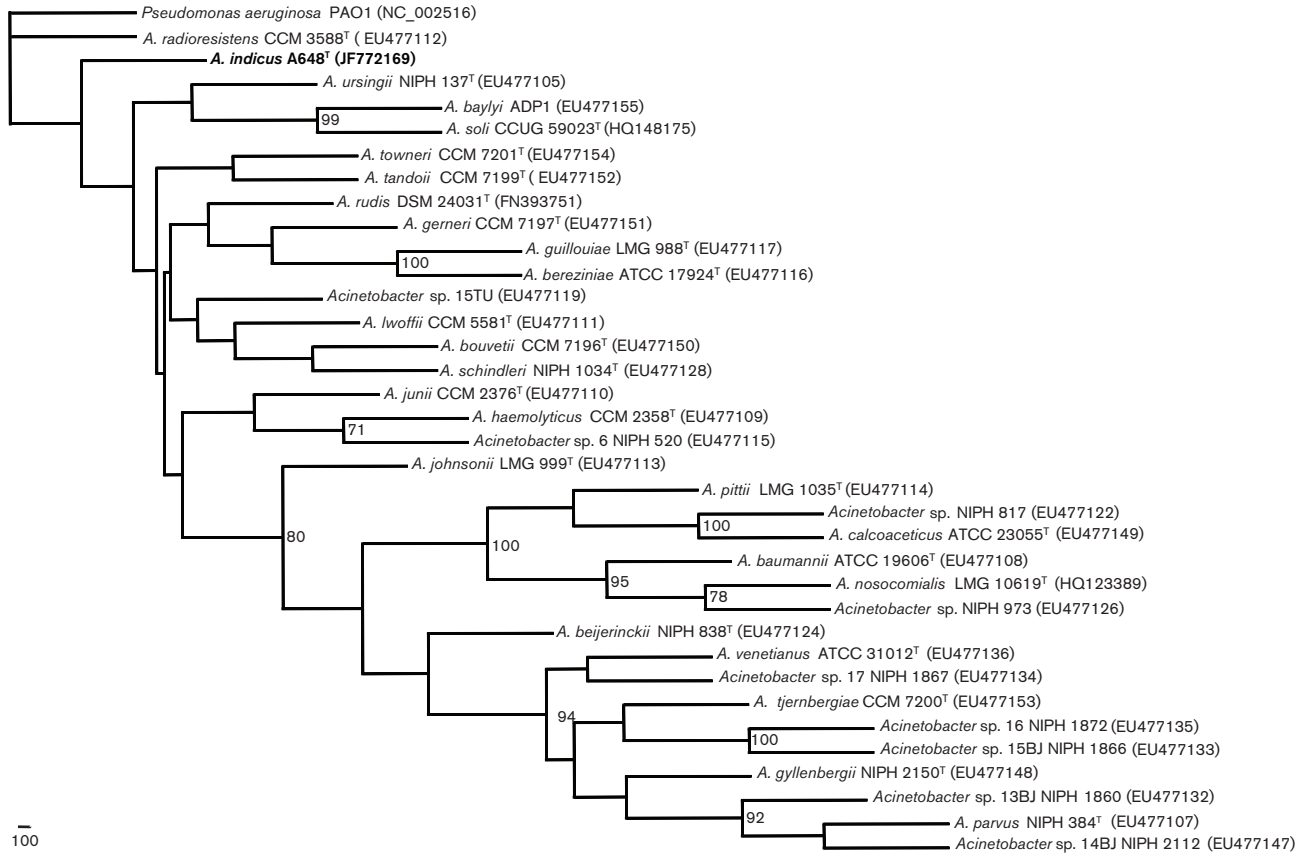

Fig. 2. Phylogenetic tree based on two concatenated variable zones of the *rpoB* gene sequences of strain A648^T and representative members of the genus *Acinetobacter*. The tree was constructed using the NJ method. Bootstrap values >70 % are indicated (based on 1000 resamplings). GenBank accession numbers 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 : 1ω8*c*	1.8	2.2	2.9	3.0	1.7	1.0	0.3
18 : 1ω9*c*	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 : 1ω7*c* and/or 16 : 1ω6*c*, summed feature 5 consists of 18 : 0 ante and/or 18 : 2ω6,9*c* and summed feature 8 consists of 18 : 1ω6*c* and/or 18 : 1ω7*c*.

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 KH_2PO_4, 5 g Na_2HPO_4, 2 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4.7H_2O$, 0.001 g $CaCl_2.2H_2O$ and 0.001 g $FeSO_4.7H_2O$ (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* (Vaneechoutte *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 H_2S 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 : 1ω9*c* and summed feature 3, comprising (16 : 1ω7*c* and/or 16 : 1ω6*c*) 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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