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\textsuperscript{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 \textit{rpoB} gene and the 16S rRNA gene sequence revealed that strain A648\textsuperscript{T} belonged to the genus \textit{Acinetobacter}. DNA–DNA relatedness values between strain A648\textsuperscript{T} and other closely related members (16S rRNA gene sequence similarity greater than 97 %), namely \textit{Acinetobacter radioresistens} DSM 6976\textsuperscript{T}, \textit{A. venetianus} ATCC 31012\textsuperscript{T}, \textit{A. baumannii} LMG 1041\textsuperscript{T}, \textit{A. parvis} LMG 21765\textsuperscript{T}, \textit{A. junii} LMG 998\textsuperscript{T} and \textit{A. soli} JCM 15062\textsuperscript{T}, were found to be less than 8 %. The major cellular fatty acids of strain A648\textsuperscript{T} were 18 : 1\textit{o}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\textsuperscript{T} indicated the presence of diphosphatidylglycerol, phosphatidylethanolamine, followed by phosphatidylglycerol and phosphatidylincholine. The predominant polyamine of strain A648\textsuperscript{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 \textit{Acinetobacter}, strain A648\textsuperscript{T} is found to be a novel species of the genus \textit{Acinetobacter}, for which the name \textit{Acinetobacter indicus} sp. nov. is proposed. The type strain is A648\textsuperscript{T} (=DSM 25388\textsuperscript{T}=CCM 7832\textsuperscript{T}).

The genus \textit{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 & Prevot, 1954). Species belonging to this genus have been split into two genomospecies (Bouvet & Grimont, 1986; Bouvet & Grimont, 1986). Members of the genus \textit{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 organic 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 \textit{x}- and \textit{\textbeta}-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 \textit{x}– and \textit{\textbeta}-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\textsuperscript{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 \textsuperscript{1}–1, 5 g yeast extract \textsuperscript{1}–1, 5 g NaCl \textsuperscript{1}–1, 1 g glucose \textsuperscript{1}–1 and 15 g agar \textsuperscript{1}–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\textsuperscript{T} was determined by the following methods:

- A comparative analysis of a partial sequence of the \textit{rpoB} gene and the 16S rRNA gene sequence revealed that strain A648\textsuperscript{T} belonged to the genus \textit{Acinetobacter}.
- DNA–DNA relatedness values between strain A648\textsuperscript{T} and other closely related members (16S rRNA gene sequence similarity greater than 97 %), namely \textit{Acinetobacter radioresistens} DSM 6976\textsuperscript{T}, \textit{A. venetianus} ATCC 31012\textsuperscript{T}, \textit{A. baumannii} LMG 1041\textsuperscript{T}, \textit{A. parvis} LMG 21765\textsuperscript{T}, \textit{A. junii} LMG 998\textsuperscript{T} and \textit{A. soli} JCM 15062\textsuperscript{T}, were found to be less than 8 %.
- The major cellular fatty acids of strain A648\textsuperscript{T} were 18 : 1\textit{o}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\textsuperscript{T} indicated the presence of diphosphatidylglycerol, phosphatidylethanolamine, followed by phosphatidylglycerol and phosphatidylincholine.
- The predominant polyamine of strain A648\textsuperscript{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).
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 A648T was found to represent a novel species of the genus *Acinetobacter*.

The 16S rRNA gene sequence of strain A648T was amplified using the 8F (5′-AGAGTTTGATCCTGCGCTCAG-3′) and 1542R (5′-AAGGAGTTGATCCAGGCGGA-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 sequence obtained was manually checked and aligned 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 A648T belonged to the genus *Acinetobacter*. The sequence similarity was calculated as the nucleotide differences between the 16S rRNA gene of A648T and those of the other members of the genus. The search revealed that the nearest neighbours of strain A648T are *Acinetobacter radioreisens* DSM 6976T (97.6%), *A. venetianus* ATCC 31012T (97.5%), *A. baumannii* LMG 1041T (97.4%), *A. parvus* LMG 21765T (97.4%), *A. junii* LMG 998T (97.3%) and *A. soli* JCM 15062T (97.0%).

The construction of the phylogenetic tree, almost full-length 16S rRNA gene sequences of all the recognized species 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 3263–3773 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 A648T 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 A648T 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 A648T 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 ± SDs are presented in Table S1). Each value was the mean of four
Stackebrandt & Goebel, 1994), strain A648T represents a
mended for the delineation of a species (Wayne
et al., 1987; Stackebrandt & Goebel, 1994), strain A648T
represents a novel species of the genus Acinetobacter.

Fatty acid methyl ester (FAME) analysis of strain A648T
and six recognized species of the genus that are the nearest
neighbours of strain A648T, namely A. radioresistens DSM 6976T,
A. venetianus ATCC 31012T, A. baumannii LMG 1041T, A. parvus
LMG 21765T, A. junii LMG 998T and A. soli JCM 15062T, was carried out. All strains were grown on
LB agar medium at 28 °C for 24 h and was then lyophilized to
obtain the dry cell mass. Polyamines were then extracted as
obtained 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 A648T were 18:1ω9c
(19.6 %), summed feature 3 (16:1ω7c and/or 16:1ω6c,
15.9 %), 16:0 (10.6 %) and 12:0 (6.4 %). Fatty acid
analysis also indicated that strain A648T 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, 18:1ω9c 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 A648T and those in the other
recognized species of this genus (Kämpfer, 1993). This
profile thus confirms that A648T 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. 1. Phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationship of strain A648T with
the other members of the genus Acinetobacter. P. immobilis ATCC 43116T (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.
Polar lipid analysis of strain A648T 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 A648T 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 \textit{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 A648T. The presence of this quinone system supported the fact that strain A648T belongs to the genus \textit{Acinetobacter} (Kim et al., 2008; Nishimura et al., 1988; Collins & Jones, 1981). All these chemotaxonomic data suggest that strain A648T represents a novel species of the genus \textit{Acinetobacter}.

The DNA G+C content of strain A648T, as determined by 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 \textit{Acinetobacter} by Vaz-Moreira et al. (2011).

The colour, size and shape of cells of strain A648T were observed on LB agar plates incubated at 28 °C for 24 h. Gram staining was performed using HiMedia Gram stains.
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 A648T was streaked on LB agar plates and incubated at 4, 10, 28, 37, 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 A648T was cultured to test its 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 A648T is a Gram-negative, non-motile, aerobic bacterium. Strain A648T appeared light yellow, convex and smooth with circular colonies after 24 h of incubation at 28 °C on LB agar. Strain A648T was negative in tests for oxidase activity and positive for catalase. Several morphological and phenotypic characteristics, as observed in this study, differentiated strain A648T from related members of the genus Acinetobacter (Tables 2 and S2). Selected phenotypic characteristics of strain A648T determined in this study were also compared with the 26 validly named species (Table 2). The study revealed that strain A648T is a non-haemolytic Acinetobacter strain, which has the ability to utilize benzoate, ethanol, D-lactate, phenylacetate, but not mannose, D-fructose, raffinose, cellobiose, xylose, mannotol, 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 A648T 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

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**Table 1.** Cellular fatty acid profile of strain A648T and the type strains of closely related members of the genus Acinetobacter (16S rRNA gene sequence similarity greater than 97 %)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:0</td>
<td>0.8</td>
<td>0.5</td>
<td>2.5</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
<td>0.4</td>
</tr>
<tr>
<td>12:0</td>
<td>6.4</td>
<td>11.2</td>
<td>5.1</td>
<td>9.7</td>
<td>5.9</td>
<td>3.8</td>
<td>10.5</td>
</tr>
<tr>
<td>13:0</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
<td>0.4</td>
<td>-</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>14:0</td>
<td>1.7</td>
<td>1.3</td>
<td>0.7</td>
<td>0.7</td>
<td>1.7</td>
<td>1.3</td>
<td>0.6</td>
</tr>
<tr>
<td>16:0</td>
<td>10.6</td>
<td>15.6</td>
<td>18.2</td>
<td>17.6</td>
<td>16.8</td>
<td>16.3</td>
<td>20.2</td>
</tr>
<tr>
<td>17:0</td>
<td>1.5</td>
<td>1.2</td>
<td>2.4</td>
<td>2.4</td>
<td>0.7</td>
<td>1.7</td>
<td>0.5</td>
</tr>
<tr>
<td>18:0</td>
<td>4.6</td>
<td>1.5</td>
<td>0.7</td>
<td>0.9</td>
<td>1.0</td>
<td>2.3</td>
<td>0.9</td>
</tr>
<tr>
<td>12:0 2-OH</td>
<td>2.2</td>
<td>0.9</td>
<td>7.6</td>
<td>4.6</td>
<td>3.9</td>
<td>7.6</td>
<td>3.9</td>
</tr>
<tr>
<td>12:0 3-OH</td>
<td>4.2</td>
<td>6.5</td>
<td>9.2</td>
<td>5.9</td>
<td>5.6</td>
<td>8.9</td>
<td>6.5</td>
</tr>
<tr>
<td>17:1 anteiso</td>
<td>1.82</td>
<td>2.2</td>
<td>2.9</td>
<td>3.0</td>
<td>1.7</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>18:1 anteiso</td>
<td>19.6</td>
<td>25.8</td>
<td>25.2</td>
<td>34.9</td>
<td>38.2</td>
<td>28.1</td>
<td>36.6</td>
</tr>
<tr>
<td>17:0</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
<td>0.2</td>
<td>-</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*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 iso and/ or 14:0 3-OH, summed feature 3 consists of 16:1 anteiso and/or 16:1 iso, summed feature 5 consists of 18:0 anteiso and/or 18:2 anteiso.

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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 (1 L): 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.

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   | 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  | 28  | 5   | 1   | 15  | 11  | 9   | 1   | 2   | 14  | 2   | 20  | 20  | 1   |
| Growth at  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 44 °C  | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | ±   | −   | +   |
| 41 °C  | +   | +   | −   | −   | −   | V   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | ±   | −   | +   | −   | −   |
| 37 °C  | +   | +   | +   | +   | +   | +   | +   | V   | +   | +   | +   | V   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   |
| Haemolytic activity | −   | +   | −   | −   | −   | +   | +   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | +   | −   | −   | −   | −   |
| Acidification of D-glucose | −   | −   | +   | +   | +   | V   | +   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| Utilization of: |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Adipate  | −   | +   | V   | +   | −   | −   | +   | V   | +   | +   | −   | NA  | −   | +   | +   | −   | +   | −   | +   | −   | −   | −   | −   | −   | −   |
| β-Alanine | −   | −   | +   | −   | −   | −   | NA  | +   | V   | +   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| 4-Aminobutyrate | D   | +   | +   | −   | −   | NA  | +   | V   | +   | +   | +   | V   | +   | +   | +   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| 1-Arginase | −   | +   | +   | −   | −   | NA  | −   | −   | −   | −   | −   | V   | +   | +   | +   | −   | −   | −   | +   | +   | −   | −   | −   | −   | −   |
| 1-Aspartate | −   | −   | +   | +   | +   | +   | +   | +   | +   | +   | +   | V   | −   | +   | +   | −   | V   | −   | −   | −   | −   | −   | −   | −   |
| Benzoate  | +   | +   | +   | −   | −   | NA  | +   | V   | +   | +   | +   | NA  | +   | +   | +   | +   | NA  | +   | V   | +   | +   | +   | +   | +   |
| 2,3-Butanediol | D   | +   | +   | −   | −   | NA  | +   | +   | −   | −   | −   | −   | −   | −   | −   | V   | +   | +   | +   | +   | −   | −   | −   | −   | −   |
| Citroconate | −   | −   | V   | −   | −   | −   | −   | NA  | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| Citrate (Simmons) | −   | +   | +   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | V   | +   | +   | +   |
| Ethanol   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| D-Glucose | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| L-Histidine | −   | +   | V   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| D-Lactate  | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   |
| D-Malate  | −   | −   | V   | +   | +   | −   | +   | +   | +   | +   | +   | −   | D   | −   | V   | +   | −   | D   | +   | NA  | V   | +   | NA  | +   | V   |
| L-Ornithine | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| Phenylacetate | +   | +   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| Putrescine | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |
| D-Ribose  | D   | NA  | V   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   | −   |

Growth at: +, 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.
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 diphasatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylcholine.

The type strain, A648T (=DSM 25388T=CCM 7832T), 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


