Gulbenkiania indica sp. nov., isolated from a sulfur spring

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A novel bacterium, designated strain HT27T, was isolated from a sulfur spring sample collected from Athamalik, Orissa, India, and was characterized by using a polyphasic approach. Cells were Gram-negative, strictly aerobic, rod-shaped and motile by means of a single polar flagellum. Strain HT27T was oxidase- and catalase-positive. Growth was observed at pH 5.0–11.0 and at 15–45 °C; the highest growth yield was observed at pH 7.5–8.0 and 30–37 °C. The G+C content of the genomic DNA of strain HT27T was 63 mol%. The major cellular fatty acids were C16 : 1ω7c (44.24 %), C16 : 0 (27.65 %), C18 : 1ω7c (13.98 %), C12 : 0 (2.60 %) and C12 : 0 3-OH (2.22 %). 16S rRNA gene sequence analysis indicated that strain HT27T clustered with the genus Gulbenkiania and showed 99.0 % similarity to Gulbenkiania mobilis E4FC31T. However, the level of DNA–DNA relatedness between strain HT27T and G. mobilis E4FC31T was 30 %. On the basis of phenotypic and chemotaxonomic characteristics, 16S rRNA gene sequence analysis and DNA–DNA hybridization data, strain HT27T is considered to represent a novel species of the genus Gulbenkiania, for which the name Gulbenkiania indica sp. nov. is proposed. The type strain is HT27T (＝DSM 17901T＝JCM 15969T).

During a study of the bacterial diversity in sediment samples collected from a sulfur spring located at Athamalik, Orissa, India, a novel strain, designated HT27T, was isolated on nutrient agar medium (HiMedia). Based on the results of the present polyphasic taxonomic study, we conclude that strain HT27T represents a novel species of the genus Gulbenkiania.

The surface temperature of the sediment from which strain HT27T was recovered was 43 °C and the pH was 7.4. A 10 g (wet weight) sediment sample was inoculated into 50 ml nutrient broth and incubated on a shaker (ISF-1-V; Adolf Kuhner AG) at 200 r.p.m. and 37 °C. After incubation overnight, the suspension was serially diluted and plated on nutrient agar. A cream-coloured colony, designated strain HT27T, was chosen for further characterization. For short-term preservation, the culture was streaked on nutrient agar and stored at 4 °C. For long-term preservation, the culture was stored at −80 °C in glycerol.

Cell morphology was examined by transmission electron microscopy (model FEI Morgagni 268D). The presence of flagella was determined by using cells that were placed onto a carbon-coated grid and negatively stained with 2 % phosphotungstic acid (Sharma et al., 1989). Oxidase activity was assayed with discs impregnated with dimethyl p-phenylenediamine (HiMedia). Catalase activity was assayed by mixing a pellet of a freshly centrifuged culture with a drop of hydrogen peroxide (10 %, v/v). Anaerobic growth was determined with a BD GasPak EZ system (Becton Dickinson). All other routine biochemical tests and procedures were performed as described by Panda et al. (2009). The pH range for growth was determined in the range 5.0–11.0 in steps of 1 pH unit. pH was adjusted by addition of HCl or NaOH. The temperature range for growth was tested in nutrient broth from 15 to 50 °C. Nutrient agar containing 1–4.5 % (w/v) NaCl was inoculated and incubated at 30 °C for 4 days to test for salt tolerance. Growth occurred at 15–45 °C; the optimum growth temperature was 30–37 °C. Growth of strain HT27T was observed at pH 5.5–11.0; the highest growth yield was observed at pH 7.5–8.0. Strain HT27T grew on nutrient agar medium containing 1.0 % (w/v) NaCl but not with 2.0 or 3.0 % NaCl.

Utilization of organic compounds other than amino acids was determined in mineral salts medium containing substrates at a concentration of 5 g l−1. The mineral salts medium contained (per litre distilled water) 4.0 g Na2HPO4, 1.5 g KH2PO4, 0.01 g CaCl2·2H2O, 1.0 g NH4Cl and 0.5 g MgSO4·7H2O (Das et al., 1996). The medium was adjusted to pH 7.0 with 4 M NaOH. Utilization of amino acids as

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HT27T is DQ415656.

A table comparing the fatty acid composition of strain HT27T and Gulbenkiania mobilis E4FC31T is available as supplementary material with the online version of this paper.
sole nitrogen and carbon sources was determined in mineral salts medium lacking \( \text{NH}_4\text{Cl} \). Amino acids were added at a concentration of 2.0 g L\(^{-1}\).

Antibiotic resistance of strain HT27\(^T\) was checked on nutrient agar containing different concentrations of antibiotics. Strain HT27\(^T\) was resistant to streptomycin (10 \( \mu \text{g ml}^{-1}\)) but was susceptible to kanamycin (50 \( \mu \text{g ml}^{-1}\)), neomycin (30 \( \mu \text{g ml}^{-1}\)), chloramphenicol (30 \( \mu \text{g ml}^{-1}\)), tetracycline (15 \( \mu \text{g ml}^{-1}\)), nalidixic acid (20 \( \mu \text{g ml}^{-1}\)), rifampicin (20 \( \mu \text{g ml}^{-1}\)) and ampicillin (40 \( \mu \text{g ml}^{-1}\)).

Cells of strain HT27\(^T\) were Gram-negative, aerobic, non-spore-forming, straight rods, 1.1 \( \mu \text{m} \) wide and 2.3 \( \mu \text{m} \) long. Cells generally occurred singly and were motile by means of a single polar flagellum (Fig. 1). Colonies on nutrient agar were smooth, mucoid, round, cream-coloured and about 1.5–2.0 mm in diameter. Single colonies appeared within 1 day at 30 °C. The phenotypic properties of strain HT27\(^T\) are given in the species description and differential characteristics between strain HT27\(^T\) and \textit{Gulbenkia mobilis} E4FC31\(^T\) are detailed in Table 1.

For estimation of the G+C content of the genomic DNA of strain HT27\(^T\), DNA was isolated according to standard methods (Sambrook & Russell, 2001). DNA was degraded enzymically into nucleotides as described by Mesbah \textit{et al.} (1989). The nucleoside mixture obtained was then separated by HPLC (Shimadzu Corp.) by using a VYDAC201 SP54 analytical column (C18, 5 \( \mu \text{m}, 250 \times 4.6 \text{ mm} \)) equipped with a guard column (201 GD54H; Vydac). Operating conditions were as described by Tamaoka & Komagata (1984), as follows: temperature, 45 °C; 10 \( \mu \text{l} \) sample; solvent, 0.3 M \((\text{NH}_4)_2\text{HPO}_4\)/acetonitrile, 40 : 1 (v/v); pH 4.4; flow rate 1.3 ml min\(^{-1}\). Non-methylated lambda phage DNA (Sigma) was used as the calibration reference. The G+C content of the genomic DNA of strain HT27\(^T\) was 63.0 mol\%, a value close to that reported for \textit{G. mobilis} E4FC31\(^T\) (63 mol\%; Vaz-Moreira \textit{et al.}, 2007).

Cellular fatty acids were analysed from cells grown on trypticase soy agar (Difco) plates for 3 days. Cells were saponified and transmethylated as described by Kuykendall \textit{et al.} (1988). Fatty acid methyl ester mixtures were separated by using the Sherlock Microbial Identification System (MIDI Inc.), via an Agilent model 6890N gas chromatograph. The major fatty acids of strain HT27\(^T\) were \( \text{C}_{16:1 \alpha 7c} \) (44.24 \%), \( \text{C}_{16:0} \) (27.65 \%), \( \text{C}_{18:1 \alpha 7c} \) (13.98 \%), \( \text{C}_{12:0} \) (2.60 \%) and \( \text{C}_{12:0 \text{3-OH}} \) (2.22 \%) (see Supplementary Table S1, available in IJSEM Online), a profile similar to that reported for \textit{G. mobilis} E4FC31\(^T\) (Vaz-Moreira \textit{et al.}, 2007).

![Fig. 1. Electron micrograph of a cell of strain HT27\(^T\), showing a single polar flagellum. Bar, 1 \( \mu \text{m} \).](image)

### Table 1. Differential characteristics between strain HT27\(^T\) and \textit{G. mobilis} E4FC31\(^T\)

Data were obtained in this study unless indicated.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HT27(^T)</th>
<th>\textit{G. mobilis} E4FC31(^T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation source</td>
<td>Sulfur spring</td>
<td>Municipal wastewater*</td>
</tr>
<tr>
<td>Cell shape</td>
<td>Straight rods</td>
<td>Curved rods*</td>
</tr>
<tr>
<td>Cell size (( \mu \text{m}, \text{mean} \pm \text{SD} ))</td>
<td>2.3 ± 0.13 x 1.1 ± 0.1</td>
<td>0.95 ± 0.17 x 0.38 ± 0.11*</td>
</tr>
<tr>
<td>Voges–Proskauer test</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Indole production</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Anaerobic growth in the presence of nitrate</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Growth on:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium succinate, sodium malate, sodium fumarate</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>4-Aminobutyric acid</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>l-Tyrosine, l-threonine, l-lysine, l-cysteine</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>l-Glutamic acid, l-leucine, l-proline</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Resistance to streptomycin (10 ( \mu \text{g ml}^{-1}))</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>5.0–11.0</td>
<td>5.5–9.0*</td>
</tr>
</tbody>
</table>

*Data from Vaz-Moreira \textit{et al.} (2007).
For 16S rRNA gene sequence analysis, DNA was isolated according to Sambrook & Russell (2001). The 16S rRNA gene of strain HT27T was amplified based on the method of Das et al. (1996). PCR products were purified by using a QIAQuick gel extraction kit and were sequenced by using a CEQ dye terminator cycle sequencing kit in a model CEQ 8000 automated DNA sequencer (Panda et al., 2009). The 16S rRNA gene sequence of strain HT27T comprised 1501 nt, and was compared with sequences in GenBank and the Ribosomal Database Project (Maidak et al., 1997) after BLAST searches (Altschul et al., 1997). Strain HT27T showed 99.0% 16S rRNA gene sequence similarity to G. mobilis E4FC31T. A neighbour-joining phylogenetic tree was constructed based on 16S rRNA gene sequences according to Kimura's two-parameter model (Kimura, 1980) by using the MEGA 3.1 (Kumar et al., 2004) software package. The statistical significance of branch points was calculated based on 1000 bootstrap resamplings of the data (Felsenstein, 1985). In the phylogenetic tree constructed, strain HT27T clustered with G. mobilis E4FC31T (Fig. 2).

DNA–DNA hybridization experiments were carried out between strain HT27T and G. mobilis E4FC31T following the method of Ezaki et al. (1989), as modified by Bhadra et al. (2008), by using a 32P-CTP-labelled probe. The level of DNA–DNA relatedness between strain HT27T and G. mobilis E4FC31T was 30%. Therefore, considering 70% DNA–DNA relatedness as the cut-off point for bacterial species delineation (Wayne et al., 1991; Stackebrandt & Goebel, 1994), strain HT27T should be regarded as representing a novel species of the genus *Gulbenkiania*.

Based on phenotypic properties, cellular fatty acid analysis, 16S rRNA gene sequence analysis and DNA–DNA relatedness data, strain HT27T is considered to represent a novel species of the genus *Gulbenkiania*, for which the name *Gulbenkiania indica* sp. nov. is proposed.

**Description of Gulbenkiania indica** sp. nov.

*Gulbenkiania indica* (in’di.ca. L. fem. adj. indica of India, the geographical origin of the type strain).

Cells are rods, 1.1 μm wide by 2.3 μm long. Cells usually occur singly, and are motile by means of a single polar flagellum. Grows at 15–45 °C and pH 5.0–11.0. Colonies on nutrient agar plates are smooth, mucoid, round and cream-coloured. Resistant to streptomycin (10 μg ml−1). Positive for catalase, oxidase and growth on MacConkey agar medium. Negative for methyl red test, Voges–Proskauer reaction, indole production, urease, H2S production, citrate utilization, anaerobic growth in the presence of nitrate, gelatin liquefaction and starch hydrolysis. Negative for assimilation of D-mannose, D-glucose, D-mannitol, D-xylene, maltose, lactose, sucrose, raffinose, D-rhamnose, D-arabinose, D-ribose, D-fructose, D-sorbitol, trehalose, sodium succinate, sodium malate, sodium fumarate, L-glutamic acid, L-leucine, L-lysine, L-alanine, L-proline, L-aspartic acid, L-tryptophan, L-phenylalanine, L-methionine, L-serine, L-valine, L-isoleucine, L-histidine and L-asparagine. Positive for assimilation of sodium acetate, inositol, 4-amino butyric acid, L-tyrosine, L-threonine, L-lysine and L-cysteine. The major fatty acids are C16:0, C18:1ω7c, C12:03-OH and C12:0 3-OH. The DNA G+C content of the type strain is 63.0 mol%. Phylogenetically, this bacterium belongs to the family *Neisseriaceae*.

The type strain, HT27T (=DSM 17901T =JCM 15969T), was isolated from a sulfur spring in Orissa, India.

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**References**


