**Shewanella chilikensis** sp. nov., a moderately alkaliphilic gammaproteobacterium isolated from a lagoon

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A Gram-negative, motile, rod-shaped, facultatively anaerobic bacterium was isolated from sediment of Chilika Lagoon, a brackish water lagoon in India. The strain, designated JC5T, was able to grow in the presence of 0–8.0 % NaCl and at pH 7.0–10.0. The isolate was positive for oxidase and catalase and exhibited α-haemolysis. The major fatty acids were iso-C15 : 0(18.3 %), C16 : 0(11.3 %), C17 : 1ω8c (13.1 %) and a summed feature, C16 : 1ω7c and/or C16 : 1ω6c (15.1 %). The genomic DNA G + C content was 54.6 mol%. A phylogenetic tree based on the 16S rRNA gene sequences showed that strain JC5T forms a lineage within the genus *Shewanella* and is closely related to *Shewanella haliotis* DW01T (98.0 %), *Shewanella algae* ATCC 51192T (97.6 %) and *Shewanella marina* C4T (95.8 %). Further, genomic DNA–DNA hybridization of strain JC5T with *S. haliotis* DW01T and *S. algae* ATCC 51192T showed relatedness of only 42 and 23 %, respectively. On the basis of phenotypic and molecular genetic evidence, strain JC5T represents a novel species of the genus *Shewanella*, for which the name *Shewanella chilikensis* sp. nov. is proposed. The type strain is JC5T (=CCUG 57101T =NBRC 105217T =KCTC 22540T).

The genus *Shewanella* is a member of the class Gammaproteobacteria (Anzai et al., 2000) and comprises a group of Gram-negative, motile, rod-shaped, oxidase-positive, non-fermentative and facultatively anaerobic aquatic and marine bacteria (Park et al., 2009; Gauthier et al., 1995; MacDonell & Colwell, 1985; Venkateswaran et al., 1999). At the time of writing, the genus *Shewanella* comprised 48 recognized species (http://www.bacterio. cict.fr/s/shewanella.html). The majority of *Shewanella* species have been isolated from aquatic and marine environments and most can grow by anaerobic respiration (Bowman, 2005). While studying the anoxygenic phototrophic bacterial diversity of Chilika Lagoon, India, strain JC5T appeared as a pale-brown colony on anaerobic agar slants used for purifying cultures of purple anoxygenic phototrophic bacteria. Strain JC5T did not contain any bacterial chlorophyll or carotenoids and analysis of its 16S rRNA gene sequence indicated that the strain belongs to the genus *Shewanella*. In this communication, we propose a novel moderately alkaliphilic species of the genus *Shewanella*.

Strain JC5T was isolated from a sediment sample taken from the southern part of Chilika Lagoon, near Barkul, Orissa, India (19° 47’ N 85° 49’ E), on 12 May 2007. The sample that yielded strain JC5T had a pH of 8.5 and salinity of 2 % (w/v). The sediment sample was originally used for the enrichment of purple bacteria in Pfennig’s mineral medium (Biebl & Pfennig, 1981) with (l-1) 3.0 g sodium pyruvate as carbon source, 0.3 g yeast extract and 0.3 g Casamino acids as growth factors and 1.2 g ammonium chloride as nitrogen source. Phototrophic incubation (2400 lx) at 30 °C for 4–5 days resulted in the formation of pale-coloured enrichments, which were streaked directly onto agar slants (25 x 150 mm test tubes sealed with butyl rubber stoppers and the gas phase replaced with argon). Pale-brown colonies appeared after 2 days and the colonies

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JC5T is FM210033.

Supplementary tables showing GN2 MicroPlate results for strain JC5T and cellular fatty acid compositions for strain JC5T and its closest phylogenetic relatives are available with the online version of this paper.
were purified by repeated streaking on agar slants. The purified culture was grown in nutrient broth and designated strain JC5T. The pure culture of strain JC5T was maintained under refrigeration at 4 °C and preserved as lyophilized cells.

Cells of strain JC5T grown on nutrient agar were observed by phase-contrast microscopy (Olympus BH-2) for shape, size and motility and by transmission electron microscopy (H-7500; Hitachi) for flagella. Utilization of organic compounds as carbon sources/electron donors was tested in Pfennig’s medium (Biebl & Pfennig, 1981) containing the specific organic compound (0.35%, w/v) in the presence of yeast extract (0.01%, w/v). Fermentative growth was tested using triple-sugar iron medium and also with the methyl red-Voges–Proskauer method.

Nitrogen source utilization was tested by replacing ammonium chloride with different nitrogen sources [sodium nitrate, sodium nitrite, urea and ammonium chloride (at 0.1%, w/v) and N2 (gas phase replaced with nitrogen in tubes sealed with butyl rubber stoppers)]. Requirements for vitamins were tested by replacing yeast extract with different vitamins [vitamin B12, biotin, niacin, p-aminobenzoic acid, pantothenate, pyridoxal phosphate, riboflavin, thiamine or a cocktail of all these vitamins (0.02%, w/v)] as growth factors. Biochemical tests (Table 1) were carried out in prescribed media to meet the requirements of the standard methods as mentioned by Cappuccino & Sherman (1998). Phylogenetically related strains (Shewanella halidiotis DW01T, S. algae ATCC 51192T and S. marina C4T) were tested under the same conditions. Growth was measured turbidometrically at 540 nm from cultures after centrifugation at 15 000 r.p.m. for 15 min and resuspension in distilled water. Other biochemical tests were performed using GN2 MicroPlates (Biolog) (Supplementary Table S1, available in IJSEM Online) according to the manufacturer’s instructions except that strains were suspended in distilled water supplemented with 2% (w/v) sea salts. Antibiotic resistance was determined using the disc diffusion method with commercial antibiotic-impregnated discs (BBL Becton Dickinson). The results were interpreted according to the guidelines set down by Clinical Laboratory Standards Institute (CLSI, 2003). Haemolytic activity was tested on trypticase soy agar supplemented with 5% (v/v) defibrinated sheep blood (Benson, 2002).

Fatty acid methyl esters were prepared from cells grown in the above medium and identified according to the instructions of the Microbial Identification System.

Table 1. Phenotypic characteristics that differentiate strain JC5T from its closest phylogenetic neighbours

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation source</td>
<td>Marine lagoon sediment</td>
<td>Salt marsh</td>
<td>Abalone</td>
<td>Seawater</td>
</tr>
<tr>
<td>Colony colour</td>
<td>Pale brown</td>
<td>Pink</td>
<td>Pink–orange</td>
<td>Pale brown</td>
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<tr>
<td>Growth at:</td>
<td></td>
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<tr>
<td>4 °C</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>42 °C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>pH range (optimum)</td>
<td>7–10 (8)</td>
<td>5–11 (7–8)</td>
<td>5–11 (7)</td>
<td>5–10 (7)</td>
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<tr>
<td>Carbon source utilization</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Malate</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Mannose</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Glucose</td>
<td>–</td>
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<td>W</td>
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<td>Casein hydrolysis</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Gelatin liquefaction</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Nitrate reduction</td>
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<td>Arginine dihydrolase</td>
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<td>Lysine decarboxylase</td>
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<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Ornithine decarboxylase</td>
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<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Urease</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Haemolysis</td>
<td>+ (2)</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Antibiotic sensitivity</td>
<td></td>
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<tr>
<td>Penicillin</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td>Vancomycin</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>54.6</td>
<td>52–54</td>
<td>53.7</td>
<td>40.8</td>
</tr>
</tbody>
</table>

*Data taken from: a, Simidu et al. (1990); b, Nozue et al. (1992) (range of values for 36 strains, including the type strain).
(Sasser, 1990). The DNA G+C content was determined by HPLC using the methods of Mesbah et al. (1989). The taxonomic relationships between strain JC5\(^T\) and \(S.\) algæ ATCC 51192\(^T\) and \(S.\) haliotis DW01\(^T\) were examined further using DNA–DNA hybridization. Genomic relatedness was determined using a membrane filter technique (Seldin & Dubnau, 1985) using a DIG High Prime DNA Labelling and Detection starter kit II (Roche).

Genomic DNA was obtained from 1–2 ml well-grown culture from nutrient broth (dehydrated medium; HiMedia) using a genomic DNA extraction kit (Qiagen). PCR amplification was performed as described by Imhoff et al. (1998) with recombinant \(Taq\) polymerase and the primers F\(^{-}\)27 (5\(^{-}\)-GTTTGAATTCCTGCTGCAAG-3\(^{-}\)) and R\(^{-}\)1489 (5\(^{-}\)-TACGTTAGCTACGACTCA-3\(^{-}\)) (positions 11–27 and 1489–1506, respectively, according to the Escherichia coli 16S rRNA gene sequence numbering system of the International Union of Biochemistry). Sequencing of the amplification products was outsourced to MWG Biotech Pvt Ltd (Bangalore, India). The resultant sequence of strain JC5\(^T\) was aligned manually against sequences obtained from the GenBank database. Phylogenetic trees were inferred from bases that were available for all sequences (positions 42–1450; \(E.\) coli numbering system) using neighbour-joining (Saitou & Nei, 1987), Fitch–Margoliash (Fitch & Margoliash, 1967) and maximum-likelihood (Guindon & Gascuel, 2003) methods. Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The resultant neighbour-joining tree topology was evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Alignment and phylogenetic analyses were carried out using the jPhyDIT program (available at http://plaza.snu.ac.kr/~jchun/jphydit/) and PAUP 4.0 (Swofford, 1998) as described by Chun et al. (2000).

On nutrient agar, aerobically grown colonies of strain JC5\(^T\) are pale brown, circular and convex with entire margins. Individual cells are rods, 1.5–2.0 \(\mu\)m long and 0.5–1.0 \(\mu\)m wide. Electron microscopy showed that the strain has a single polar flagellum. Substrates that were utilized as carbon sources/electron donors under organoheterotrophic conditions are given in Table 1. Ammonium chloride, nitrate and glutamate were used as nitrogen sources for growth, while nitrite and molecular nitrogen were not utilized. Addition of NaCl to the medium was not required for growth of strain JC5\(^T\), but the strain could tolerate NaCl up to a concentration of 8.0 %. Strain JC5\(^T\) could grow at pH 7.0–10.0 (optimum pH 8.0) (Fig. 1) and at 25–42 °C (optimum 28–30 °C). There is no vitamin requirement for strain JC5\(^T\); however, addition of yeast extract (0.01 %, w/v) enhanced growth. Strain JC5\(^T\) was catalase-positive and \(\alpha\)-haemolytic. Starch was not hydrolysed; casein was hydrolysed. Gelatin was not liquefied and indole was not produced from \(L\)-tryptophan. Although lipase and acid phosphatase tests were negative, strain JC5\(^T\) showed urease activity. Acid formation from glucose and lactose was negative. These results show clear phenotypic differences from recognized \(Shewanella\) species (Table 1).

The whole-cell fatty acid profile of strain JC5\(^T\) (Supplementary Table S2) included \(C_{12}:0\) (3.5 %), \(C_{16}:0\) (11.3 %), iso-\(C_{13}:0\) (4.3 %), iso-\(C_{15}:0\) (18.3 %), \(C_{17}:1\)\(\beta\)8c (13.1 %) and summed feature 4 (\(C_{16}:0\)7c and/or \(C_{16}:1\)\(\beta\)6c; 15.1 %). Significant differences in the contents of predominant fatty acids between strain JC5\(^T\) and type strains of related species were found: e.g. summed feature 4 was found only in strain JC5\(^T\) and summed feature 3 was found only in the type strains of other \(Shewanella\) species (although both summed features include \(C_{16}:1\)\(\omega\)7c). The DNA G+C content of strain JC5\(^T\) was 54.6 mol% (HPLC), which is similar to values reported for \(S.\) algæ and \(S.\) haliotis.

Preliminary sequence comparison with 16S rRNA gene sequences held in GenBank indicated that our isolate was related closely to the genus \(Shewanella\). After manual alignment of the newly determined sequence (1442 nt) with those of representatives of the genus \(Shewanella\), strain JC5\(^T\) showed highest 16S rRNA gene sequence similarity to sequences from \(S.\) haliotis DW01\(^T\) (98.0 %) and \(S.\) algæ ATCC 51192\(^T\) (97.6 %). To elucidate the phylogenetic relationship between the isolate and other species of the genus \(Shewanella\), phylogenetic trees were constructed using three different tree-making algorithms. The neighbour-joining tree (Fig. 2) showed that strain JC5\(^T\) formed a distinct branch with the clade comprising \(S.\) haliotis DW01\(^T\) and \(S.\) algæ ATCC 51192\(^T\), and this was also observed in the Fitch–Margoliash tree (not shown).

Genomic DNA–DNA hybridization of strain JC5\(^T\) with \(S.\) haliotis DW01\(^T\) and \(S.\) algæ ATCC 51192\(^T\) showed relatedness values of only 42 and 23 %, respectively. Thus, levels of genetic relatedness according to
DNA–DNA hybridization experiments were less than 70 %, which supports the conclusion that strain JC5T represents a novel and distinct species.

On the basis of the phylogenetic, genomic and phenotypic data, it is clear that strain JC5T represents a novel species within the genus Shewanella (Bowman, 2005), for which the name Shewanella chilikensis sp. nov. is proposed.

**Description of Shewanella chilikensis sp. nov.**

*Shewanella chilikensis* (chi.li.ken’sis. N.L. fem. adj. chilikensis pertaining to Chilikia, referring to the isolation of the type strain from Chilika lagoon, Orissa, India).

On nutrient agar, aerobically grown colonies are pale brown, circular and convex with entire margins. Cells are rod-shaped, 1.5–2.0 μm long and 0.5–1.0 μm wide and motile by single polar flagellum. Gram-negative, non-fermentative, non-spore-forming and facultatively anaerobic. Moderately alkaliphilic (range pH 7.0–10.0, optimum pH 8.0). Growth occurs with 0–8 % (w/v) NaCl and at 25–42 °C (optimum 28–30 °C). No vitamin requirement but yeast extract enhances growth. Oxidase- and catalase-positive. Negative for nitrate reduction and glucose fermentation. Produces urease and ornithine decarboxylase, but not lipase, arginine dihydrolase or lysine decarboxylase. Produces H2S but not indole. Hydrolyses casein but not gelatin or starch. Acid production from carbohydrates (glucose, lactose) and acid phosphatase activity are negative. The carbon sources that are utilized include fumarate, malate, pyruvate, succinate, glycogen, Tweens 40 and 80, N-acetylgalactosamine, N-acetylglucosamine, pyruvic acid methyl ester, acetic acid, cis-aconitic acid, citric acid, formic acid, α- and β-hydroxybutyric acid, α-ketoacetic acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, propionic acid, succinic acid, bromosuccinic acid, L-alanine, L-α-lactyl glycine, L-glutamic acid, glycyl L-aspartic acid, glycyl
l-glutamic acid, l-leucine, l-proline, l-serine, l-threonine, inosine, uridine and thymidine. Citrate is not utilized. Exhibits α-haemolysis. The predominant fatty acids (>5%) are iso-C_{15:0} C_{16:0}, C_{17:1}ω6c and summed feature 4 (C_{16:1}ω7c and/or C_{16:1}ω6c). The DNA G+C content of the type strain is 54.6 mol% (HPLC).

The type strain, JCS\textsuperscript{T} (=CCUG 57101\textsuperscript{T} =NBRC 105217\textsuperscript{T} =KCTC 22540\textsuperscript{T}), was isolated from sediment of Chilika Lagoon, Orissa, India.

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References


