Ancylobacter rudongensis sp. nov., isolated from roots of Spartina anglica

Yu Hua Xin,1 Yu Guang Zhou,1 Hui Ling Zhou1 and Wen Xin Chen2

1China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, P.R. China
2China Agricultural University, Beijing 100094, P.R. China

A curved, ring-like bacterium, strain AS 1.1761T, isolated from the roots of Spartina anglica, was studied by a polyphasic approach. According to phylogenetic analysis, strain AS 1.1761T belongs to the genus Ancylobacter, with 99–21% 16S rDNA sequence similarity to Ancylobacter aquaticus, the only species described so far in this genus. However, strain AS 1.1761T had no significant DNA–DNA binding with the type strain of A. aquaticus. In addition, strain AS 1.1761T differed from A. aquaticus in many phenotypic features. Based on molecular and phenotypic data, a novel species, Ancylobacter rudongensis sp. nov., is proposed. The type strain is AS 1.1761T (=JCM 11671T).

The genus Microcyclus was created by Ørskov (1928) for bacteria with a ring-like shape; such bacteria had been isolated from wood-like waters, freshwater ponds and occasionally from soil. Because of their mostly aquatic habitat, Ørskov named them Microcyclus aquaticus (Raj, 1981). Other bacteria with a similar morphology had been described; Gromov (1963) proposed ‘Microcyclus major’, which was isolated from Scenedesmus sp., an algae collected from lake water in Leningrad. Raj (1970) described ‘Microcyclus flavus’, which was isolated from a deep, freshwater well in Long Beach, California, in 1964. Consequently, in the eighth edition of Bergey’s Manual of Determinative Bacteriology, this genus included three species: M. aquaticus, ‘M. flavus’ and ‘M. major’ (Staley, 1974). However, on the basis of phenotypic characteristics and genomic DNA G+C content, ‘M. flavus’ became a heterotypic synonym of Spiroreta lingualae and ‘M. major’ was transferred to a novel genus, Flectobacillus, as Flectobacillus major (Claus et al., 1968; Larkin et al., 1977). A novel species that was isolated from a marine environment, ‘Microcyclus marinus’, was described by Raj (1976); because of its similarity to F. major, Borrall & Larkin (1978) reclassified it into the genus Flectobacillus as ‘Flectobacillus marinus’. After further studies, Raj & Maloy (1990) proposed that ‘F. marinus’ should be transferred to the novel genus Cyclobacterium. At the time of writing, the genus Microcyclus comprises only one species, M. aquaticus (Staley & Konopka, 1984).

The genus Microcyclus had been recognized formally from 1928 to 1983. However, the same name had been used before Ørskov for some fungi, one of which had been known as a plant pathogen for a long time (Saccardo, 1905). To avoid confusion and to conform to the rules of the Bacteriological Code (Lapage et al., 1975), Raj (1983) suggested that the name Microcyclus should be replaced by Ancylobacter.

In this study, we describe a curved, ring-like bacterium, strain AS 1.1761T, which was isolated from the roots of Spartina anglica on the beach of Rudong County, Jiangsu Province, P.R. China. Phylogenetic analysis indicated that it was a member of the genus Ancylobacter. DNA–DNA hybridization with DNA from Ancylobacter aquaticus DSM 101T revealed that it represented a novel genospecies. Phenotypic analysis showed that this genospecies could be differentiated clearly from A. aquaticus and, consequently, a novel species, Ancylobacter rudongensis sp. nov., is proposed.

S. anglica is a grass that grows on beaches, originally in Europe. It can protect beaches and dykes from damage by waves, and its stalks and leaves can be used as fodder, green manure and fuel. It is now planted widely on beaches in China. Some tender roots (3–5 cm) of S. anglica were sterilized with 0–1% HgCl2 and washed with sterile water. After grinding in a mortar, the juice was spread-plated onto Döbereiner nitrogen-free agar medium (Döbereiner et al., 1976) that contained 0–3% NaCl. A ring-like bacterium was isolated and deposited in the China General Microbiological Culture Collection Center under the accession number AS 1.1761T. A. aquaticus DSM 101T was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). To study its morphological characteristics, strain AS 1.1761T was incubated at 30°C in Ancylobacter–Spiro-soma medium (DSMZ, 1998). Staining procedures were performed as described by Doetsch (1981). Inoculum was incubated in Döbereiner nitrogen-free
medium that contained 0.2% agar to test for nitrogenase ability by the acetylde 
(C₂H₂) reduction method, as described by Hanson & Phillips (1981). To test utilization 
of carbon sources, a basal medium [containing 0.2% 
(NH₄)₂SO₄, 0.05% NaH₂PO₄·H₂O, 0.05% K₂HPO₄, 0.02% MgSO₄·7H₂O and 0.01% CaCl₂·2H₂O] was supple-
mented with vitamins, as described by Green & Bousfield (1982). Production of acid or gas from carbohydrates 
was determined by using the method of Hugh & Leifson (1953). DNAS was tested as described by Schreier (1969) 
and the KCN test was performed as described by Farmer et al. (1980). The following characteristics were tested as 
described by Smibert & Krieg (1981): indole production, methyl red and Voges–Proskauer tests, nitrate reduction 
and denitrification, H₂ production, milk reactions, starch hydrolysis, gelatin liquefaction, ascusul hydrolysis, urease, 
lipase (Tweek 80), arginine dihydrolase, phenylalanine deaminase, ornithine decarboxylase, arginine decarboxylase 
and lysine decarboxylase.

Chromosomal DNA was extracted by using a modified version of the method of Marmur (1961). The 16S rRNA 
gene was amplified by PCR using the following primers: 5′-CGggatccAGAGTTTGATCCGTCAGACAGGAACGCT 
CT-3′ and 5′-CGggatccTACGGCTACCTTGTTACGACTTAAGCTTCACCCC-3′ (positions 8–37 and 1479–1506, respectively, 
according to Escherichia coli 16S rDNA sequence numbering). Small letters indicate BamHI recognition sequences. 
Amplified products were purified and ligated into plasmid pUC18. Recombinants were transformed into 
Escherichia coli DH 5α (Sambrook et al., 1989). DNA from clones was purified by using the Wizard Plus Minipreps DNA Purification 
system (Promega). Automated sequencing was carried out by using an ABI PRISM BigDye Terminator Cycle 
Sequencing Ready Reaction kit (Perkin Elmer) on an ABI PRISM 377XL DNA sequencer (Applied Biosystems). The 
16S rDNA sequence of strain AS 1.1761T was aligned manually by using the CLUSTAL X program (version 1.64b; 
Thompson et al., 1997) against corresponding sequences 
that were retrieved from GenBank. Evolutionary distance 
matrices were calculated by employing programs within the 
PHYLIP package (Felsenstein, 1993). An unrooted phylo-
genetic tree was constructed according to the neighbour-
joining method (Saitou & Nei, 1987). GenBank/ 
EMBL accession numbers of reference sequences are given after strain names. Numbers at branch-points represent confidence levels from 1000 replicate bootstrap resamplings. Bar, 
2 nucleotide substitutions per 100 positions.

![Fig. 1. Phylogenetic tree of strain AS 1.1761T and related taxa, based on 16S rDNA sequence comparison by using the neighbour-joining method (Saitou & Nei, 1987). GenBank/EMBL accession numbers of reference sequences are given after strain names. Numbers at branch-points represent confidence levels from 1000 replicate bootstrap resamplings. Bar, 2 nucleotide substitutions per 100 positions.](image)

DNA G+C content was determined by thermal denatura-
tion (Marmur & Doty, 1962). E. coli K-12 was used as the reference. DNA–DNA hybridization between strains AS 
1.1761T and A. aquaticus DSM 101T was determined on the basis of the initial DNA–DNA reassociation rate 
method, as described by De Ley et al. (1970). Renaturation 
was achieved at 75 °C. The procedure was performed on a 
model 1 Bio 20 UV/VIS spectrometer equipped with a 
temperature program controller (Perkin Elmer).

The almost-complete 16S rDNA sequence of strain AS 
1.1761T (1457 bp) was compared with those of related 
strains (Fig. 1). The result showed that strain AS 1.1761T 
was closest to A. aquaticus DSM 101T, with 99.21% 16S 
rDNA sequence similarity. This indicated that strain AS 
1.1761T should be assigned to the genus *Ancylobacter*. The 
DNA G+C content of strain AS 1.1761T was 68.2 mol%, 
which is within the range of the genus *Ancylobacter* (66.3– 
68.8 mol%) (Raj, 1981). No (0%) DNA–DNA hybridization 
was found between isolate AS 1.1761T and *A. aquaticus* 
DSM 101T and, consequently, strain AS 1.1761T can be 
considered as a distinct genospecies of the genus *Ancylobacter*.

Phenotypically, strain AS 1.1761T can be differentiated from *A. aquaticus* by several characteristics (Table 1). Based on 
these genomic and phenotypic data, a novel species of the 
genus *Ancylobacter* is proposed, with the name *Ancylobacter rudongensis* sp. nov.

**Description of Ancylobacter rudongensis** sp. nov.

*Ancylobacter rudongensis* (ru.dong.en’sis. N.L. masc. adj. rudongensis referring to Rudong County, Jiangsu Province, P.R. China, where the strain was isolated).

Gram-negative curved rods, 0.6–0.8 μm in diameter, 
non-motile, without capsule and gas vesicles. Intracellular 
granules of poly-β-hydroxybutyrate are formed. Colonies 
are white, convex, round, opaque, entire and 0.5–1.0 mm 
in diameter after 7 days incubation on *Ancylobacter– 
Spirosoma* agar. Optimum temperature for growth is 28– 
30 °C; no growth occurs at 4 or 45 °C. Optimum pH for 
growth is 6.8–7.0; no growth occurs at pH 5.5 or 10.0. 
Growth occurs in the presence of 0–5% (w/v) NaCl. 
Microaerophilic in semi-solid, nitrogen-free medium to fix 
free nitrogen. Delayed growth occurs in the presence of 
KCN. Fluorescent pigment is not produced. Urease-, 
arginine decarboxylase- and lysine decarboxylase-positive. 
Arginine dihydrolase, phenylalanine deaminase, ornithine
Table 1. Differential phenotypic characteristics of strain AS 1.1761^T and A. aquaticus

Data for A. aquaticus DSM 101^T were taken from Raj (1976), Larkin et al. (1977), Raj (1981) and Holt et al. (1994). Both taxa can utilize formate and methanol. ND, Not determined.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>A. rudongensis AS 1.1761^T</th>
<th>A. aquaticus DSM 101^T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape</td>
<td>Curved rods</td>
<td>Curved rods</td>
</tr>
<tr>
<td>Encapsulated</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Poly-β-hydroxybutyrate present</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Growth at 37 °C</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Growth in presence of 3 % NaCl</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Nitrogenase</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Lysine decarboxylase</td>
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<td>−</td>
</tr>
<tr>
<td>Ornithine decarboxylase</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Aesculin hydrolysis</td>
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<td>−</td>
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<td>Acid production from:</td>
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</tr>
<tr>
<td>Melibiose</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Salicin</td>
<td>+</td>
<td>−</td>
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<td>Cellobiose</td>
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<td>−</td>
</tr>
<tr>
<td>Malote</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>−</td>
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<td>Utilization of:</td>
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</tr>
<tr>
<td>Fumarate</td>
<td>+</td>
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<tr>
<td>2-Oxoglutarate</td>
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<td>Adonitol</td>
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</tr>
<tr>
<td>Malote</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Habitat</td>
<td>Roots of Spartina anglica</td>
<td>Soil, freshwater</td>
</tr>
</tbody>
</table>

decarboxylase, DNase and lipase (Tween 80) are negative. Alkali is produced from litmus milk. H₂S and indole are not produced. Methyl red and Voges–Proskauer tests are negative. Nitrates are reduced. Growth does not occur anaerobically with nitrate. Aesculin and gelatin are hydrolysed, but starch is not. The following carbohydrates are used as sole carbon sources: starch, maltose, methyl α-D-glucoside, adonitol, glycerol, glucose, ribose, inulin, L-arabinose, fructose, galactose, mannitol, xylose, aesculine, sorbitol, sodium gluconate, α-ketoglutaric acid, alanine, proline, sodium acetate, fumaric acid, sodium malonate, sodium citrate, sodium succinate, sodium lactate, sodium malate, sodium propionate, methanol, sodium formate, sodium pyruvate, amaroid, sucrose, dextrin and cellobiose; trehalose, sorbose, raffinose, melibiose, mannose, lactose, dulcitol, inositol, meso-erythritol, rhamnose, melizitose, arginine, sodium tartrate, sodium hippurate and salicin are not used. Acid is formed from mannose, ribose, glycerol, mannitol, sorbitol, galactose, cellobiose, rhamnose, salicin, glucose, maltose, L-arabinose, trehalose, xylose, melibiose, adonitol, fructose and lactose, but not from inositol, raffinose, inulin, melizitose, starch, glycogen, methyl α-D-glucoside, sucrose, sorbose, sodium gluconate, dulcitol, meso-erythritol or dextrin. No gas is formed from these carbohydrates. DNA G+C content is 68-2 mol%.

The type strain is AS 1.1761^T (= JCM 11671^T), which was isolated from the roots of Spartina anglica in Rudong County, Jiangsu Province, P.R. China.

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References


