Spirosoma rigui sp. nov., isolated from fresh water

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A Gram-negative, yellow-pigmented bacterium capable of gliding motility, designated strain WPCB118^T, was isolated from fresh water collected from the Woopo wetland (Republic of Korea). Cells were rod-shaped and sometimes filamentous. The major fatty acids were iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (45.6%), C_{16:1}ω5c (18.5%), iso-C_{15:0} (9.5%) and C_{16:0} (8.8%). The predominant menaquinone and polar lipid were MK-7 and phosphatidylethanolamine, respectively. The DNA G+C content was 53.3 mol%. A phylogenetic tree based on 16S rRNA gene sequences showed that strain WPCB118^T had an evolutionary lineage within the radiation encompassing the members of the family 'Flexibacteraceae', its closest neighbour being *Spirosoma linguale* LMG 10896^T (93.7% gene sequence similarity). Data from this polyphasic study indicated that strain WPCB118^T could not be assigned to any recognized species. Strain WPCB118^T represents a novel species of the genus *Spirosoma*, for which the name *Spirosoma rigui* sp. nov. is proposed. The type strain is WPCB118^T (=KCTC 12531^T=NBRC 101117^T).

The family *Flexibacteraceae* constitutes a phylogenetically diverse group within the phylum *Bacteroidetes*. One of the lineages in this group comprises the genera *Spirosoma*, *Runella*, *Larkinella* and *Dyadobacter* currently classified in the family 'Flexibacteraceae'. The genus *Spirosoma* currently contains a single species, *Spirosoma linguale* (Larkin & Borrall, 1984). Strains of *S. linguale* have been isolated from soil or fresh water. The cells of *S. linguale* typically appear as helices or long filaments, but they exhibit variations of shape and size under different cultural conditions.

In the course of our study on wetland microbial diversity, a rod-shaped bacterial strain, designated strain WPCB118^T, was isolated from a freshwater sample and was the subject of a taxonomic investigation. On the basis of evidence from this polyphasic taxonomic investigation, strain WPCB118^T represents a novel species of the genus *Spirosoma*.

Strain WPCB118^T was isolated from a freshwater sample, collected from the wetland of Woopo (35° 33' N 128° 25' E) in the Republic of Korea during July 2000, by using the standard dilution plating technique. Isolation was achieved

using PYGV (Staley, 1968) and R2A agar (Oxoid) at 25 °C for 7 days. The isolate was routinely cultured on R2A agar and maintained as a glycerol suspension (20%, w/v) at –80 °C.

Bacterial DNA preparation and PCR amplification and sequencing of the 16S rRNA gene were carried out as described previously (Chun & Goodfellow, 1995). The resultant sequence of strain WPCB118^T was aligned manually against sequences obtained from the GenBank database. Phylogenetic trees were inferred from the regions available for all sequences (positions 31–1472; *Escherichia coli* numbering system) using the Fitch–Margoliash (Fitch & Margoliash, 1967) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The resultant neighbour-joining tree topology was evaluated by means of bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Alignment and phylogenetic analyses were carried out using the PHYDIT program (available at <http://plaza.snu.ac.kr/~jchun/phydit/>) and PAUP 4.0 (Swofford, 1998) as described previously (Chun *et al.*, 2000).

Preliminary sequence comparisons with 16S rRNA gene sequences held in GenBank indicated that our isolate was related to the family 'Flexibacteraceae'. The newly determined sequence was then aligned manually against representatives of the family 'Flexibacteraceae' on the basis of bacterial 16S rRNA secondary structure. Strain

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain WPCB118^T is EF507900.

Phase-contrast and scanning electron micrographs of cells of strain WPCB118^T are available as a supplementary figure with the online version of this paper.

WPCB118^T showed the highest level of 16S rRNA gene sequence similarity with *S. linguale* LMG 10896^T (93.7 %) followed by *Larkinella insperata* LMG 22510^T (85.4 %). This distant relationship between the novel isolate and *S. linguale* LMG 10896^T was also supported in the phylogenetic trees. When strain WPCB118^T was analysed together with all recognized species of the genera in the family 'Flexibacteraceae', it was shown to represent a distinct subline within the genus *Spirosoma* in all trees inferred in this study (Fig. 1). On the basis of 16S rRNA gene sequence similarity data and the results of the phylogenetic analysis, it is clear that our isolate represents a novel genomic species within the genus *Spirosoma*.

Growth was tested on various standard bacteriological media such as MS agar (containing, 1⁻¹, 1.0 g peptone, 1.0 g yeast extract, 1.0 g glucose and 15.0 g agar), nutrient agar (Difco), tryptone soy agar (TSA; Oxoid) and R2A agar. Cells grown at 30 °C for 2 days were observed using a differential interference microscope (BX50; Olympus) and a scanning electron microscope (JSM 5410LV; JEOL). Cells of strain WPCB118^T grown on TSA at 30 °C for 3–7 days were used for the physiological and biochemical tests. Motility was examined by observing cells grown in wet mounts, using a phase-contrast microscope (Axioskop 40; Zeiss). The following growth conditions were checked, using TSA for up to 1 week: pH range (pH 4–12), temperature range (4–42 °C), tolerance of NaCl (0, 1, 2, 3, 5, 10%, w/v) and growth in an anaerobic chamber (CO₂/H₂/N₂, 10:10:80; Sheldon Manufacturing). Catalase and oxidase activities were investigated using 3 % (v/v) hydrogen peroxide and Kovács' reagent (Kovács, 1956), respectively. Other physiological and biochemical tests were performed using API 20E, API 20NE, and API 50 CH kits (bioMérieux). The pigment absorption spectrum between 250 and 700 nm was analysed by using an Ultrospec 2100pro UV/visible spectrophotometer (Amersham Biosciences) following

ethanol extraction (Gosink *et al.*, 1998). The presence of flexirubin-type pigments was investigated as described by Bernardet *et al.* (2002).

Enzyme activities were tested using an API ZYM kit according to the manufacturer's instructions. Antibiotic resistance was determined with the disc diffusion method. The results were interpreted according to the guidelines set forth by the National Committee for Clinical Laboratory Standards (NCCLS, 2003).

The cells of strain WPCB118^T were found to be rod-shaped and 0.8–1.0 × 1.5–10.8 µm in size. They can also appear as filaments up to 54 µm long (see Supplementary Fig. S1, in IJSEM Online). The coils and helices that are typical of *S. linguale* LMG 10896^T (Larkin & Borrall, 1984) were not produced by strain WPCB118^T. Detailed results from the physiological and biochemical analyses are given in Table 1 and in the species description. Pigment extracts of strain WPCB118^T showed an absorption maximum at 451 nm and a shoulder at 478 nm. Flexirubin-type pigments were absent.

It is evident that there are several phenotypic characteristics that serve to distinguish strain WPCB118^T from the phylogenetically related strain *S. linguale* LMG 10896^T, e.g. differences in the production of oxidase and alkaline phosphatase and in the utilization of particular carbohydrates and susceptibility to antibiotics (Table 1).

Cellular fatty acids of strain WPCB118^T were analysed as methyl esters by GLC according to the instructions of the Microbial Identification System (MIDI). Cellular fatty acid profiles of strain WPCB118^T and its closest phylogenetic neighbour (*S. linguale* LMG 10896^T) are presented in Table 2.

Isoprenoid quinones were extracted according to the method of Minnikin *et al.* (1984) and analysed by HPLC as described previously (Kroppenstedt, 1985). Strain WPCB118^T contained MK-7 as the predominant menaquinone. Polar lipids

Fig. 1. Neighbour-joining phylogenetic tree, based on almost-complete 16S rRNA gene sequences, showing relationships between strain WPCB118^T and members of the family 'Flexibacteraceae'. Bootstrap percentage (based on neighbour-joining analyses of 1000 resampled datasets) are shown at the nodes. *Bacteroides fragilis* ATCC 25285^T (X83935) was used as an outgroup. Bar, 0.1 nucleotide substitution per position.

Table 1. Characteristics that differentiate strain WPCB118^T from its closest phylogenetic neighbour, *S. linguale* LMG 10896^T

Data are from Larkin & Borrall (1984). All species are positive for gliding motility, catalase and β -galactosidase. All species are negative for Gram stain, hydrolysis of gelatin, H₂S production, indole and acetoin production, reduction of nitrate to nitrite and utilization of L-fucose, sorbose, dulcitol, glycerol, inositol, mannitol, sorbitol, citrate and *N*-acetylglucosamine. All species are sensitive to ampicillin (10 μ g), erythromycin (15 μ g), streptomycin (10 μ g) and tetracycline (30 μ g), but resistant to polymyxin B (300 U). +, Positive; –, negative; R, resistant; S, sensitive.

Characteristic	WPCB118 ^T	<i>S. linguale</i> LMG 10896 ^T
Cell morphology	Rod-shaped cells and filaments can be present	Ring-like and horseshoe-shaped cells, filaments, coils and helices can be present
Temperature range for growth (°C)	4–37	5–39
Optimum temperature (°C)	30	20–30
NaCl range for growth (% w/v)	0–1	0–1.25
Colony morphology	Circular, opaque, convex, smooth, wet, slimy	Circular, convex, entire margin
Oxidase	–	+
Alkaline phosphatase activity (API ZYM test)	–	+
Assimilation of:		
Cellobiose	–	+
Fructose	–	+
Galactose	–	+
Maltose	–	+
Mannose	–	+
Melibiose	–	+
Raffinose	–	+
Rhamnose	–	+
Starch	–	+
D-Xylose	–	+
Antibiotic susceptibility:		
Gentamicin (10 μ g)	R	S
Kanamycin (30 μ g)	R	S
DNA G + C content (mol%)	53.3	51–53

were separated by TLC according to the method of Minnikin *et al.* (1975). Individual lipids were identified with several spray reagents (Embley & Wait, 1994) and through co-running with authentic standards (Sigma). Aminolipids were detected by applying a solution of ninhydrin and then molybdophosphoric acid on the same TLC plate. The polar lipids of strain WPCB118^T were phosphatidylethanolamine, an unknown aminolipid and an unknown lipid. The lipid profile of strain WPCB118^T also included a small amount of phosphatidylglycerol.

The DNA G + C content of strain WPCB118^T, determined using the thermal denaturation method of Marmur & Doty (1962), was found to be 53.3 mol%.

Phylogenetic, genomic, chemotaxonomic and phenotypic data clearly indicate that strain WPCB118^T represents a novel species of the genus *Spirosoma*, for which the name *Spirosoma rigui* sp. nov. is proposed.

Description of *Spirosoma rigui* sp. nov.

Spirosoma rigui (ri'gu.i. L. gen. n. *rigui* of a well-watered place, referring to the site of isolation, the Woopo wetland, Korea).

Cells are rod-shaped, Gram-negative, do not form endospores and are motile by gliding. Non-diffusible yellow

pigments are produced. Negative for cytochrome oxidase and positive for catalase. Facultatively anaerobic. Colonies on R2A agar are opaque, convex, circular, smooth, wet and slimy and approximately 2 mm in diameter after 4 days at 30 °C (pH 7). Cells in R2A broth sometimes appear filamentous, forming filaments up to 54 μ m long. Cells grow best on media such as R2A, MS and PCA, but grow weakly on nutrient agar and TSA. Growth occurs at pH 6–11 (optimum, pH 7) and at 4–37 °C (optimum, 30 °C). Growth occurs in 0–1 % (w/v) NaCl (optimum, 0 %). Flexirubin-type pigments are absent. Does not produce H₂S or acetoin. Produces *N*-acetyl- β -glucosaminidase, α -glucosidase, aesculin hydrolase, β -galactosidase, leucine arylamidase and valine arylamidase. Does not produce alkaline phosphatase, cystine arylamidase, α -chymotrypsin, esterase (C4), esterase lipase (C8), α -fucosidase, α -galactosidase, β -glucuronidase, β -glucosidase, lipase (C14), α -mannosidase, naphthol-AS-BI-phosphohydrolase or trypsin. Utilizes aesculin and glucose as sole carbon and energy sources. Does not utilize following substrates: arabinose, D-fucose, β -gentiobiose, lactose, lyxose, maltose, mannose, melezitose, ribose, sucrose, D-tagatose, trehalose, turanose, xylose, arabitol, erythritol, mannitol, xylitol, methyl α -D-glucoside, methyl α -D-mannoside, methyl β -D-xyloside, amygdalin, arbutin, glycogen, inulin, salicin, *N*-acetylglucosamine, adipate, caprate, gluconate, 2-ketogluconate, 5-ketogluconate, malate or phenyl acetate. Cells are

Table 2. Fatty acid content (%) of strain WPCB118^T and its closest phylogenetic neighbour, *S. linguale* LMG 10896^T

–, Not detected; tr, trace amount (<1%).

Fatty acid	WPCB118 ^T	<i>S. linguale</i> LMG 10896 ^T
Straight-chain saturated		
C _{14:0}	1.3	–
C _{15:0}	–	–
C _{16:0}	8.8	7.2
C _{18:0}	1.2	–
C _{16:0} 3-OH	2.8	4.7
Branched saturated		
iso-C _{13:0}	–	3.8
iso-C _{15:0}	9.5	7.6
iso-C _{15:0} 3-OH	2.6	4.9
iso-C _{16:0} 3-OH	–	–
iso-C _{17:0} 3-OH	3.5	6.5
anteiso-C _{15:0}	1.3	tr
Monounsaturated		
C _{16:1} ω5c	18.5	17.5
C _{18:1} ω7c	1.5	–
Summed feature 3*	45.6	47.9

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system; summed feature 3 contained iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

sensitive to the following antibiotics (μg per disc, unless otherwise indicated): amikacin (30), chloramphenicol (30), nalidixic acid (30), penicillin (10 U) and vancomycin (30). Other physiological and biochemical characteristics are given in Table 1. Major fatty acids are iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (45.6%), C_{16:1}ω5c (18.5%), iso-C_{15:0} (9.5%) and C_{16:0} (8.8%); the complete fatty acid content is given in Table 2. The predominant menaquinone is MK-7. The main polar lipid is phosphatidylethanolamine. The DNA G+C content is 53.3 mol%.

The type strain, WPCB118^T (=KCTC 12531^T=NBRC 101117^T), was isolated from fresh water from the Woopo wetland, Republic of Korea.

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