Rudanella lutea gen. nov., sp. nov., isolated from an air sample in Korea

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An orange-coloured bacterial strain, designated 5715S-11^T, was isolated from an air sample in Suwon, Republic of Korea. The strain was strictly aerobic, Gram-negative, non-spore-forming, non-flagellated and rod-shaped, frequently forming filaments. Growth of the strain was observed at 4–40 °C (optimum, 30 °C), pH 6.0–8.0 (optimum, pH 7.0) and 0–2 % (w/v) NaCl. Phylogenetically, strain 5715S-11^T was shown to be a member of the family *Spirosomaceae* within the phylum *Bacteroidetes*. Its closest relatives were *Spirosoma linguale* LMG 10896^T (87.9 % 16S rRNA gene sequence similarity) and *Larkinella insperata* LMG 22510^T (86.2 % 16S rRNA gene sequence similarity). Predominant cellular fatty acids were summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{16:1}ω5c and iso-C_{15:0}. Major polar lipids were phosphatidylethanolamine and unknown aminolipids and polar lipids. On the basis of the evidence from this polyphasic study, strain 5715S-11^T is considered to represent a novel species of a new genus, for which the name *Rudanella lutea* gen. nov., sp. nov. is proposed. The type strain of *Rudanella lutea* is 5715S-11^T (=KACC 12603^T =DSM 19387^T).

The family *Spirosomaceae* was first proposed by Larkin & Borrall (1978). Members of the family are characterized as Gram-negative, non-motile, strictly aerobic, catalase- and oxidase-positive bacteria that produce water-insoluble pigments. As originally defined, the family *Spirosomaceae*, within the phylum *Bacteroidetes*, comprised the genera *Spirosoma*, *Flectobacillus* and *Runella*. Another member of the family *Spirosomaceae*, the genus *Larkinella*, was proposed by Vancanneyt *et al.* (2006). The genus *Spirosoma*, members of which were isolated from fresh-water and soil, includes yellow-coloured, gliding bacteria (Larkin & Borrall, 1984). The genus *Larkinella*, comprising just *Larkinella insperata* isolated from water, includes pale-pink-pigmented, gliding bacteria (Vancanneyt *et al.*, 2006). Cells of species of these two genera are ring-like or horseshoe-shaped.

We isolated strain 5715S-11^T from an air sample, and this strain was shown to be phylogenetically related to

Spirosoma and *Larkinella*. In the present study, we elucidated the genotypic and phenotypic properties of strain 5715S-11^T.

An air sample was taken outside in a highly built-up area of Suwon city, Republic of Korea. The air sample was collected with an MAS-100 air sampler (Merck; single-stage multiple-hole impactor) equipped with a Petri dish containing R2A agar (BBL) emended with 200 µg cycloheximide ml⁻¹ (Sigma). The Petri dish was incubated at 30 °C for 5 days, and colonies were selected and purified by using the streak plate method. The pure culture of strain 5715S-11^T was transferred to R2A medium for further analysis. Cell morphology was examined by using phase-contrast microscopy (AXIO; Zeiss) and transmission electron microscopy with cells grown for 3 days. For transmission electron microscopy, cells were negatively stained with 0.5 % (w/v) uranyl acetate and examined with a LEO model 912AB electron microscope. The effects of NaCl concentration, temperature and pH on growth were determined at 0, 1, 2, 3 and 5 % (w/v) NaCl, 5, 10, 15, 20, 25, 30, 35, 40 and 45 °C and pH 4, 5, 6, 7, 8, 9 and 10 (buffered with citrate-phosphate buffer or Tris/HCl) (Breznak & Costilow, 1994). The methods of Smibert & Krieg (1994) were used to test the following basic

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 5715S-11^T is EF635010.

Cellular fatty acid profiles of strain 5715S-11^T, *Spirosoma linguale* DSM 74^T and *Larkinella insperata* LMG 22510^T are available as supplementary material with the online version of this paper.

physiological and biochemical characteristics: Gram reaction, oxidase and catalase reaction, deamination of phenylalanine and hydrolysis of casein, DNA, hypoxanthine, starch, Tween 80 and xanthine. Additional hydrolysis tests were also conducted by using 0.1 % (w/v) CM-cellulose, 1 % (w/v) chitin from crab shells, 0.5 % (w/v) pectin and 0.5 % (w/v) tyrosine. Flexirubin-type pigments were detected based on a colour shift after exposure to a 20 % (w/v) KOH solution (Reichenbach, 1992). Gliding motility was observed by oil-immersion phase-contrast microscopy of the edge of colonies after cultivation on R2A at 30 °C for 2 days. Anaerobic growth was checked by using a BBL anaerobic jar (Becton Dickinson). Growth on peptone water was checked by using 0.2 % Bacto peptone (Difco) in distilled water. The strain was also characterized via API 20NE, API ID 32GN and API ZYM test strips (bioMérieux), according to the manufacturer's recommendations. Cells of strain 5715S-11^T were strictly aerobic, non-motile and rod-shaped, frequently forming filaments (0.7–1 µm wide and 2.5–50 µm long) (Fig. 1). Colonies were orange-coloured, convex and circular with clear margins. The strain was able to grow on R2A and nutrient agar (Difco), but not on trypticase soy agar (Difco) or MacConkey agar (Difco). Additional phenotypic characteristics that could be used to differentiate between strain 5715S-11^T and the type strains of its closest phylogenetic neighbours (*Spirosoma linguale* and *L. insperata*; see below) are shown in Table 1.

The following chemotaxonomic characteristics were analysed: fatty acids (according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System; Sasser, 1990), phospholipids (Minnikin *et al.*, 1984) and G + C content of the DNA (Mesbah *et al.*, 1989). Dominant fatty acids were summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 29.4 %), C_{16:1}ω5c (17.7 %), iso-C_{15:0} (14.3 %), iso-C_{17:0} 3-OH (9.7 %) and anteiso-C_{15:0}

Fig. 1. Transmission electron micrograph of cells of strain 5715S-11^T grown on R2A for 3 days. Bar, 5 µm.

(7.7 %) (see Supplementary Table S1, available in IJSEM Online). The polar lipid profile of strain 5715S-11^T consisted of phosphatidylethanolamine, several unknown aminolipids and polar lipids and one unknown phospholipid (Fig. 2). The DNA G + C content of strain 5715S-11^T was 55 mol%.

Genomic DNA was isolated by the method of Ausubel *et al.* (1987). The 16S rRNA gene sequence of strain 5715S-11^T was analysed as described by Kwon *et al.* (2003). The sequence obtained was compared against reference 16S rRNA gene sequences available in the GenBank database. Phylogenetic analysis was carried out by using the MEGA 3 program (Kumar *et al.*, 2004), after multiple alignments of the data via the CLUSTAL W program (Thompson *et al.*, 1994). Phylogenetic dendrograms were constructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with bootstrap values based on 1000 replications. According to BLAST search results, strain 5715S-11^T was shown to be a member of the family *Spirosomaceae*. It shared highest 16S rRNA gene sequence similarities (87.9 and 86.2 %, respectively) with *S. linguale* LMG 10896^T and *L. insperata* LMG 22510^T. It showed less than 83 % 16S rRNA gene sequence similarity to the type strains of all other recognized species examined. In a neighbour-joining tree based on 16S rRNA gene sequences, strain 5715S-11^T formed a compact cluster with *S. linguale* LMG 10896^T and *L. insperata* LMG 22510^T with high bootstrap support (100 %; Fig. 3). A tree based on maximum-parsimony analysis revealed a similar result.

In conclusion, strain 5715S-11^T can be differentiated clearly from its closest phylogenetic neighbours, *S. linguale* and *L. insperata*. 16S rRNA gene sequence analysis showed that strain 5715S-11^T showed very low sequence similarities (≤87.9 %) to the type strains of *S. linguale* and *L. insperata*, much lower than the threshold level that is generally used to define a new genus (Ludwig *et al.*, 1998). Cells of strain 5715S-11^T lacked the morphology (ring-like, horseshoe-shaped, coiled or helical) characteristic of cells of *S. linguale* and *L. insperata*. The polar lipid profile of strain 5715S-17^T lacked aminophospholipid APL1, aminolipid AL3 and phospholipid PL1, the major components found for *S. linguale* and *L. insperata* (Fig. 2). Strain 5715S-11^T could also be differentiated from *S. linguale* and *L. insperata* on the basis of several physiological properties, including substrate assimilation patterns (Table 1). Therefore, we suggest that strain 5715S-11^T represents a novel species of a new genus, for which the name *Rudanella lutea* gen. nov., sp. nov. is proposed.

Description of *Rudanella* gen. nov.

Rudanella (Ru.da.nel'la. L.L. dim. suff. -ella; N.L. fem. dim. n. *Rudanella* arbitrary name, after RDA, Rural Development Administration, where taxonomic studies of this taxon were conducted).

Cells are strictly aerobic, Gram-negative, non-motile, non-spore-forming rods, frequently forming filaments.

Table 1. Differential phenotypic characteristics between strain 5715S-11^T, *Spirosoma linguale* and *Larkinella insperata*

Strains: 1, strain 5715S-11^T; 2, *S. linguale* DSM 74^T; 3, *L. insperata* LMG 22510^T. Data were obtained in the present study unless indicated. All three strains were positive for β -galactosidase and negative for indole production, glucose fermentation, arginine dihydrolase and urease. All strains were negative for assimilation of D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. All were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -glucosidase, *N*-acetyl- β -glucosaminidase and β -mannosidase, but negative for lipase (C14), β -glucuronidase and α -fucosidase. +, Positive; (+), weakly positive; –, negative.

Characteristic	1	2	3
Isolation source	Air	Freshwater, soil*	Water†
Colony colour	Orange	Yellow	Pale pink
Cell morphology	Straight rods and filaments	Ring-like and horseshoe-shaped; filaments, coils and helices may be present*	Ring-like and horseshoe-shaped†
Growth on peptone water	(+)	–	–
Nitrate reduction	–	+	–
Hydrolysis of:			
Aesculin	–	+	+
Gelatin	–	+	+
Starch	+	(+)*	–†
Assimilation of (API 20NE):			
D-Glucose	–	+	+
L-Arabinose	–	+	–
D-Mannose	–	+	+
N-Acetylglucosamine	–	+	+
Maltose	–	+	+
Enzymic activities (API ZYM)			
Trypsin	–	+	+
α -Chymotrypsin	–	+	+
α -Galactosidase	–	+	+
β -Galactosidase	–	+	+
α -Glucosidase	–	+	+
DNA G + C content (mol%)	55	51–53*	53†

*Data for the species from Larkin & Borrall (1984).

†Data from Vancanneyt *et al.* (2006).

Fig. 2. Two-dimensional thin-layer chromatographs of polar lipid extracts obtained from strains 5715S-11^T (a), *Spirosoma linguale* DSM 74^T (b) and *Larkinella insperata* LMG 22510^T (c) sprayed with anisaldehyde. L1–L11, Unidentified polar lipids; AL1–AL6, unidentified aminolipids; PL1–PL4, unidentified phospholipids; APL, unidentified aminophospholipid.

Fig. 3. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain 5715S-11^T and some members of the phylum *Bacteroidetes*. Asterisks indicate that the corresponding branches were also recovered in the maximum-parsimony tree. Bar, 0.02 changes per nucleotide position.

Oxidase- and catalase-positive. Nitrate is not reduced. No flexirubin-type pigments are produced. Predominant cellular fatty acids are summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{16:1}ω5c and iso-C_{15:0}. Major polar lipids are phosphatidylethanolamine and unknown aminolipids and polar lipids. Phylogenetically, the genus belongs to the family *Spirosomaceae* within the phylum *Bacteroidetes*. The type species is *Rudanella lutea*.

Description of *Rudanella lutea* sp. nov.

Rudanella lutea (lu'te.a. L. fem. adj. *lutea* orange-coloured).

Cells frequently form filaments that are 0.7–1 μm wide and 2.5–50 μm long. Colonies are orange-coloured, convex and circular with clear margins after 3 days cultivation on R2A. The pH range for growth is 6.0–9.0, with an optimum at pH 7.0. The temperature range for growth is 5–40 °C, with an optimum at 30 °C. Growth occurs at NaCl concentrations of 0–2 % (w/v). No gliding motility is observed. Hydrolyses casein, tyrosine, Tween 80 and starch, but not chitin, CM-cellulose, DNA, hypoxanthine, pectin or xanthine. In API 20NE test strips, cells are positive only for β-galactosidase. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, aesculin hydrolysis and gelatin hydrolysis. In API 20NE and API ID 32GN test strips, assimilates only glycogen. In API ZYM tests, cells are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucosidase, *N*-acetyl-β-glucosaminidase and α-mannosidase. Negative for lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase and α-fucosidase. The DNA G+C content of the type strain is 55 mol% (HPLC).

The type strain, 5715S-11^T (=KACC 12603^T =DSM 19387^T), was isolated from an air sample from Suwon City, Republic of Korea.

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