Fibrella aestuarina gen. nov., sp. nov., a filamentous bacterium of the family *Cytophagaceae* isolated from a tidal flat, and emended description of the genus *Rudanella* Weon *et al.* 2008

Manuela Filippini,¹ Miroslav Svercel,¹ Endre Laczko,² Andres Kaech,³ Urs Ziegler³ and Homayoun C. Bagheri¹

Correspondence

Homayoun C. Bagheri
bagheri@ieu.uzh.ch

¹Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

²Functional Genomics Center Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

³Center for Microscopy and Image Analysis, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

A Gram-staining-negative, pink bacterium, designated strain BUZ 2^T, was isolated from coastal mud from the North Sea (Fedderwardersiel, Germany). Cells were rod-shaped and able to form multicellular filaments. Growth after 7 days was observed at 10–40 °C, at pH 6–8 and with 0–0.5 % NaCl. The phylogenetic tree based on 16S rRNA gene sequences indicated that strain BUZ 2^T is a member of the family *Cytophagaceae*, its closest neighbours being *Rudanella lutea* 5715S-11^T, *Spirosoma linguale* LMG 10896^T and *Spirosoma panaciterrae* Gsoil 1519^T (87.8, 86.4 and 86.1 % sequence similarity, respectively). The major fatty acids were summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{16:1}ω5c and iso-C_{15:0}. The predominant respiratory quinone was MK-7 and the major polar lipids were phosphatidylethanolamine and several unidentified aminophospholipids. The DNA G + C content was 56.5 mol%. On the basis of this polyphasic study, we propose that strain BUZ 2^T represents a novel genus and species, for which the name *Fibrella aestuarina* gen. nov., sp. nov. is proposed. The type strain of *Fibrella aestuarina* is BUZ 2^T (=DSM 22563^T =CCUG 58136^T). An emended description of the genus *Rudanella* is also proposed.

The family *Cytophagaceae* (Ludwig *et al.*, 2008) constitutes a phylogenetically diverse group within the phylum *Bacteroidetes*, comprising *Spirosoma* (Baik *et al.*, 2007; Finster *et al.*, 2009; Ten *et al.*, 2009), *Runella* (Larkin & Williams, 1978), *Flectobacillus* (Larkin *et al.*, 1977), *Larkinella* (Vancanneyt *et al.*, 2006; Kulichevskaya *et al.*, 2009) and *Rudanella* (Weon *et al.*, 2008) among other genera.

Strain BUZ 2^T was isolated from a mud sample from tidal flats in Fedderwardersiel (53° 35' 59" N 8° 21' 23" E, elevation 0 m), on the North Sea coast of Germany. This strain was isolated using a selective nutrient agar containing kanamycin (50 µg ml⁻¹), developed by Flint (1985) for the isolation and enumeration of *Flavobacterium* strains. Colonies were selected according to their colour. The strain

was cultivated in liquid *Spirosoma* medium (SM; DSMZ medium 7) and R2A broth (Reasoner & Geldreich, 1985) and preserved as a glycerol suspension (1:1 mixture of glycerol and bacterial culture) at –80 °C.

Bacterial genomic DNA was extracted using the protocol described in Svercel *et al.* (2009). The 16S rRNA gene was amplified by PCR using the universal bacterial primers 27F and 1525R in a Techne TC 512 thermal cycler (Barloworld Scientific). Sequencing was performed both in house (ABI 3730 Sequencer) and at an external facility (Microsynth AG, Balgach, Switzerland). Sequences were assembled and edited manually using the Geneious Pro 3.6.2 software. Phylogenetic analysis was performed with the software package MEGA version 4.1 (Kumar *et al.*, 2001) following a multiple alignment of data with CLUSTAL_X2 (Thompson *et al.*, 1997). Phylogenetic trees were constructed by using the neighbour-joining, minimal evolution, maximum-parsimony and maximum-likelihood algorithms. Evolutionary distances were computed using the Kimura-2 model and the maximum composite likelihood method.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BUZ 2^T is GQ141052.

Two supplementary figures are available with the online version of this paper.

Maximum-likelihood analysis was performed using Garli (Zwickl, 2006). These analyses were performed using 1000 bootstrap resamplings.

According to BLAST results based on a 1319 bp fragment of the 16S rRNA gene, strain BUZ 2^T is a member of the family *Cytophagaceae*. It shares the highest 16S rRNA gene sequence similarity with *Rudanella lutea* 5715S-11^T (87.9%), *Spirosoma linguale* LMG 10896^T (86.4%) and *Spirosoma panaciterrae* Gsoil 1519^T (86.1%). These values are significantly lower than the threshold level of 95% that is generally used to delineate a potential new genus (Ludwig *et al.*, 1998). *Rudanella lutea* DSM 19387^T and *Spirosoma linguale* DSM 74^T were selected as reference strains and studied in parallel to the new isolate in all phenotypic tests. In the neighbour-joining tree based on 16S rRNA gene sequences, strain BUZ 2^T formed a cluster with *R. lutea* 5715S-11^T with high bootstrap support (Fig. 1), in agreement with the other tree-calculating methods employed (not shown).

The Gram reaction was determined using the KOH test as described by Tindall *et al.* (2007). Bacterial cell morphology and gliding motility (Bernardet *et al.*, 2002) were observed under a light microscope (Olympus BX-51) from liquid cultures grown in SM for 1 day. For transmission (Philips CM100; FEI) and scanning (Supra 50 VP; Zeiss) electron microscopy, strain BUZ 2^T was grown for 4 days in SM broth at 30 °C. Cells for transmission electron microscopy were high-pressure frozen (EMPact2; Leica Microsystems) and freeze-substituted with anhydrous acetone with 2% OsO₄ (EMAFS2; Leica) before embedding in Epon (Sigma). For scanning electron microscopy, cells were chemically fixed with 2.5% glutaraldehyde in PBS and post-fixed with 2% OsO₄, dehydrated with a graded ethanol series, critical-point dried and coated with 5 nm platinum. The presence of flagella was also assessed by transmission electron microscopy after negative staining with 2% aqueous phosphotungstic acid.

Physiological and biochemical tests were performed at 30 °C unless otherwise indicated. Catalase activity was determined by adding a drop of 3% H₂O₂ to colonies. Oxidase activity was assessed using the bioMérieux kit according to the manufacturer's protocol. Flexirubin-type

pigments were detected based on a colour shift after exposure to a 20% (w/v) KOH solution (Reichenbach, 1992).

Pigments were extracted with 99% ethanol according to Gosink *et al.* (1998). The absorption spectrum between 350 and 800 nm was recorded with a spectrophotometer (SpectraMax 384 Plus; Molecular Devices) after incubation for 10 min in the dark.

Growth was tested in R2A, SM, nutrient broth (NB; Becton Dickinson), 0.2% peptone water and 0.5% yeast water and on trypticase soy agar (TSA; Becton Dickinson), yeast extract tryptone agar (YT agar; Becton Dickinson), blood agar and MacConkey agar. Since strain BUZ 2^T grew well in R2A and SM broths, these two media were used for further analysis. Physiological and biochemical properties of strain BUZ 2^T and the two reference strains were determined using API 20NE, API 20E, API 50CH and API ZYM strips (bioMérieux) according to the manufacturer's instructions. The API 50CH strips were inoculated with cells suspended in AUX medium (bioMérieux). API 20NE, API 20E and API 50CH strips were inspected after 3 and 7 days at 30 °C, whereas the incubation time for API ZYM was 5 h at 37 °C. The temperature range and optimum temperature for growth were determined in R2A broth by growing the cultures at 4, 10, 15, 20, 30, 37 and 40 °C. The pH range and optimum pH for growth were examined in SM broth adjusted to pH 5.0–11.0 (at 1 pH unit intervals) using KH₂PO₄ and K₂HPO₄ prior to autoclaving. Higher pH was reached with additional NaOH. The pH was verified after autoclaving with pH strips. Growth at different temperatures and pH was monitored for up to 1 week. Additionally, tolerance of NaCl was tested on R2A plates adjusted to 0–10% NaCl (at 0.5% intervals). Anaerocult C (Merck) was used to generate an oxygen-depleted (5–7% O₂) and carbon dioxide-enriched (8–10% CO₂) atmosphere in anaerobe jars. Growth was followed for up to 1 week. Hydrolysis of starch, xylan, CM-cellulose, pectin, amylopectin, agar and agarose was tested by adding 0.5% of the respective polysaccharide to nutrient medium (Tindall *et al.*, 2007) gellified using gellan gum. A single streak of the test organism was made across the centre of each plate. After 3–5 days of incubation, the plates were

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain BUZ 2^T with representative members of the family *Cytophagaceae*. Bootstrap values (expressed as percentages of 1000 replications) greater than 70% are shown at branch nodes. Bar, 0.02 substitutions per nucleotide position. Accession numbers are given in parentheses. The tree was rooted using *Rhodothermus marinus* DSM 4252^T as the outgroup.

Table 1. Phenotypic properties of strain BUZ 2^T and its closest neighbours

Data were obtained in this study unless indicated. In API ZYM strips, all three strains were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -glucosidase, α -chymotrypsin and *N*-acetyl- β -glucosaminidase activities and negative for lipase (C14) and β -glucuronidase activities. None of the strains exhibited gliding motility. In API 20NE and API 20E strips, all strains were negative for indole and H₂S production, glucose fermentation, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and urease activities, nitrate reduction, citrate utilization and assimilation of D-mannitol, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid; all strains were positive for aesculin hydrolysis and β -galactosidase activity. In API 50CH strips, all strains were negative for assimilation of glycerol, erythritol, D-arabinose, L-xylose, D-adonitol, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, xylitol, D-tagatose, D- and L-fucose, D- and L-arabitol and potassium 5-ketogluconate. All strains were positive for aesculin assimilation (API 50CH) and amylopectin hydrolysis. All strains were resistant to amikacin, colistin, gentamicin, kanamycin and polymyxin B and sensitive to amoxicillin, amoxicillin/clavulanic acid, chloramphenicol, ciprofloxacin, penicillin G, rifampicin, streptomycin and tetracycline. +, Positive; w, weakly positive; -, negative.

Characteristic	BUZ 2 ^T	<i>R. lutea</i> DSM 19387 ^T	<i>S. linguale</i> DSM 74 ^T
Cell morphology	Rods, filaments	Rods, filaments	Ring-like, horseshoe-shaped
Colony colour	Pink	Orange	Yellow
Temperature range (°C)	10–40	10–40	4–37
pH range (optimum)	6–8 (7)	6–11 (7)	6–8 (8)
Tolerance of NaCl (%)	0–0.5	0–2	0–1.25
Growth on peptone	–	w	–
Oxidase activity	–	–	+
Hydrolysis of:			
Gelatin	+	–	–
Starch	+	w	w
CM-cellulose	+	–	–
Agar	+	–	–
Xylan	+	+	–
Casein	+	+	–
Agarose	+	–	–
Assimilation of (API 20NE and 50CH):			
D-Glucose	+	–	+
L-Arabinose	+	–	w
D-Mannose	+	–	+
N-Acetylglucosamine	+	–	+
Maltose	+	–	+
D-Fructose	+	–	+
Raffinose	+	–	+
Potassium gluconate	w	–	–
Methyl β -D-xylopyranoside	+	–	–

Table 1. cont.

Characteristic	BUZ 2 ^T	<i>R. lutea</i> DSM 19387 ^T	<i>S. linguale</i> DSM 74 ^T
D-Lyxose	–	–	+
Glycogen	+	–	–
Arbutin	+	–	–
D-Ribose	+	–	–
D-Xylose	+	–	+
D-Galactose	+	–	+
Methyl α -D-mannopyranoside	+	–	+
Methyl α -D-glucopyranoside	+	–	+
Amygdalin	w	–	+
Salicin	+	–	+
Cellobiose	+	–	+
Lactose	+	–	+
Melibiose	+	–	+
Sucrose	+	–	+
Trehalose	+	–	+
Inulin	+	–	+
Melezitose	+	–	+
Starch	+	–	+
Gentiobiose	+	–	w
Turanose	+	–	+
Potassium 2-ketogluconate	w	–	–
Enzyme activity (API ZYM)			
α -Fucosidase	+	–	–
α -Mannosidase	+	–	+
α -Glucosidase	+	–	+
α -Galactosidase	+	–	+
β -Galactosidase	+	–	+
Susceptibility to vancomycin	–	+	+
DNA G + C content (mol%)	56.5	55 ^{a*}	51–53 ^b

*Data taken from: *a*, Weon *et al.* (2008); *b*, Larkin & Borrall (1984) (range of values for four strains).

flooded with iodine solution. A positive test result was indicated by a colourless area around the cell growth. Hydrolysis of casein was tested on milk agar prepared according to the Sigma recipe (15 g agar, 10 ml fresh milk, 5 g peptone and 1 g yeast extract l⁻¹). A hole was made in the centre of the milk agar and subsequently filled with cell suspension in 0.7% NaCl. The plates were inspected for clear zones around the hole after 2 and 5 days of incubation.

Susceptibility to antibiotics was tested using the disc-diffusion method on R2A plates. The results were interpreted according to the guidelines set forth by the CLSI (2008). The following antibiotics (all from bioMérieux) were tested (μ g per disc unless indicated): amikacin (30),

amoxicillin (10), amoxicillin/clavulanic acid (20/10), chloramphenicol (30), ciprofloxacin (5), colistin (30 U), gentamicin sulfate (10), kanamycin (30), penicillin G (10 U), polymyxin B (300 U), rifampicin (30), streptomycin (10), tetracycline (30) and vancomycin (30).

Strain BUZ 2^T produced a pink pigment with absorbance peaks at 482 and 506 nm, with the major peak at 482 nm, whereas the major peak and shoulder of the closest relative, *S. linguale* DSM 74^T, were at 452 and 480 nm, respectively. *R. lutea* DSM 19387^T showed a major peak at 476 nm and shoulders at 452 and 506 nm (see Supplementary Fig. S1, available in IJSEM Online). Absorption between 380 and 550 nm probably results from the presence of carotenoid pigments (Zscheile *et al.*, 1942).

The phenotypic characteristics of strain BUZ 2^T are given in Table 1 and in the genus and species descriptions. In contrast to Finster *et al.* (2009), we did not observe gliding motility for *S. linguale* DSM 74^T.

For fatty acid analysis, strain BUZ 2^T, *R. lutea* DSM 19387^T and *S. linguale* DSM 74^T were grown in SM broth for 3 days at room temperature. Fatty acids were analysed by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Quinone and polar lipid analyses and determination of the DNA G + C content of strain BUZ 2^T were also carried out by the DSMZ and Dr B. J. Tindall. The quinone content of *R. lutea* DSM 19387^T was also determined in this study. Extraction and purification of genomic DNA of strain BUZ 2^T was done following the protocol of Cashion *et al.* (1977) and the G + C content was measured by HPLC (Mesbah *et al.*, 1989).

The cellular fatty acid profiles of strain BUZ 2^T, *S. linguale* DSM 74^T and *R. lutea* DSM 19387^T are shown in Table 2. The dominant fatty acids of strain BUZ 2^T were summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 33.1 %), C_{16:1}ω5c (17.5 %), iso-C_{15:0} (11.9 %), C_{16:0} (9.8 %) and iso-C_{17:0} 3-OH (9.3 %). Although the overall fatty acid composition of the three strains was rather similar, strain BUZ 2^T could be distinguished from its closest phylogenetic neighbours by a smaller proportion of summed feature 3, a larger proportion of iso-C_{16:0} and the presence of iso-C_{17:0}, iso-C_{17:1}ω9c and anteiso-C_{17:0}.

Menaquinone 7 (MK-7) was the only respiratory quinone found in strain BUZ 2^T, in agreement with previous observations for members of the genus *Spirosoma*. MK-7 was also the major respiratory quinone in *R. lutea* DSM 19387^T, but a small amount of MK-6 was also found.

The major polar lipid of strain BUZ 2^T was phosphatidylethanolamine, but several unidentified aminolipids, phospholipids and aminophospholipids were also detected (Supplementary Fig. S2). Overall, this polar lipid profile was more similar to that of *S. linguale* DSM 74^T than to

Table 2. Fatty acid content of strain BUZ 2^T and its closest neighbours

Strains: 1, BUZ 2^T; 2, *R. lutea* DSM 19387^T; 3, *S. linguale* DSM 74^T. Values are percentages of total fatty acids; components amounting to <1 % of the total fatty acids in all three strains are not shown. tr, Trace amounts (<1 %); ND, not detected. Data are from this study; bacteria were grown in SM broth at room temperature for 3 days.

Fatty acid	1	2	3
Straight-chain			
C _{15:0}	1.0	1.1	tr
C _{16:0}	9.8	4.3	3.7
Branched saturated			
iso-C _{13:0}	ND	ND	2.0
iso-C _{15:0}	11.9	8.3	7.9
iso-C _{16:0}	1.3	1.3	tr
iso-C _{17:0}	3.4	tr	tr
anteiso-C _{15:0}	2.1	1.8	2.0
anteiso-C _{17:0}	1.7	tr	tr
Hydroxy			
iso-C _{15:0} 3-OH	1.3	2.3	2.6
C _{16:0} 3-OH	1.6	3.6	2.2
iso-C _{17:0} 3-OH	9.3	6.8	7.5
Monounsaturated			
C _{16:1} ω5c	17.5	21.2	18.1
iso-C _{17:1} ω9c	1.2	tr	tr
Summed feature 3*	33.1	42.0	46.1

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

that of *R. lutea* DSM 19387^T (Weon *et al.*, 2008), but strain BUZ 2^T differed from these strains in the number of each type of polar lipid identified.

The DNA G + C content of strain BUZ 2^T was 56.5 mol%, slightly higher than the values reported for the two reference strains (Weon *et al.*, 2008; Larkin & Borrall, 1984).

Based on our analyses, we propose that strain BUZ 2^T represents a novel genus and species, for which the name *Fibrella aestuarina* gen. nov., sp. nov. is proposed. On the basis of the new data obtained in this study, an emended description of the genus *Rudanella* is also proposed.

Description of *Fibrella* gen. nov.

Fibrella (Fib.rel'la. L. fem. n. *fibra* a fibre or filament; L. dim. suff. *-ella*; N.L. fem. n. *Fibrella* a small fibre, referring to the ability of the type species to produce filaments).

Cells are facultatively anaerobic, Gram-staining-negative, non-motile rods that form multicellular filaments. Catalase-positive and oxidase-negative. Nitrate is not reduced. Flexirubin-type pigments are not 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, iso-C_{15:0},

C_{16:0} and iso-C_{17:0} 3-OH. The major polar lipid is phosphatidylethanolamine; several unidentified aminolipids, phospholipids and aminophospholipids also occur. The only respiratory quinone is MK-7. The G + C content of the type strain of the type species is 56.5 mol%. Phylogenetically, the genus belongs to the family *Cytophagaceae* within the phylum *Bacteroidetes*. The type species is *Fibrella aestuarina*.

Description of *Fibrella aestuarina* sp. nov.

Fibrella aestuarina (aes.tu.a.ri'na. L. n. *aestuarium* a tidal marsh; N.L. fem. adj. *aestuarina* belonging to a marsh, referring to the location where the type strain was isolated).

The following properties are in addition to those given in the genus description. After 1 day of incubation in SM broth, cells are usually 0.8 µm wide and 1.1–6.9 µm long, but multicellular filaments up to 245 µm long also occur (Fig. 2). During the transient growth phase in liquid culture, multicellular filaments up to 400 µm long may be formed. Colonies on R2A are pink, convex and circular with regular edges. Flagellar and gliding motility are absent. Growth occurs in R2A, SM, NB and 0.5 % yeast water and on YT agar and TSA but not on blood agar or MacConkey agar, or in peptone water. Grows at pH 6.0–8.0, with an optimum at pH 7.0. The temperature range for growth is 10–40 °C, with an optimum at 25–30 °C. Does not require NaCl for growth but can tolerate 0.5 % (w/v) NaCl. Agar, agarose, amylopectin, casein, CM-cellulose, starch and xylan are hydrolysed (showing clear haloes around the colonies). Pectin is not hydrolysed. In the API 20E strip, negative results are observed for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and urease activities, citrate utilization and H₂S and indole production. In the API ZYM strip, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, α-glucosidase, 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, α-mannosidase and α-fucosidase activities are present; lipase (C14) and

β-glucuronidase activities are absent. In the API 20NE strip, positive reactions are observed for β-galactosidase activity, aesculin and gelatin hydrolysis and assimilation of arabinose, glucose, maltose, mannose and N-acetylglucosamine; potassium gluconate is weakly assimilated and negative reactions are observed for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease activities and assimilation of D-mannitol, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. In the API 50CH strip after 7 days of incubation, the following substrates are assimilated: L-arabinose, D-ribose, D-xylose, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose (bovine origin), melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, gentiobiose, turanose and potassium 2-ketogluconate; potassium gluconate is weakly assimilated and the following substrates are not assimilated: glycerol, erythritol, D-arabinose, L-xylose, D-adonitol, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, xylitol, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol and potassium 5-ketogluconate. Resistant to amikacin (30 µg), colistin (30 U), gentamicin (10 µg), kanamycin (30 µg), polymyxin B (300 U) and vancomycin (10 µg). Sensitive to amoxicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), penicillin G (10 U), rifampicin (30 µg), streptomycin (30 µg) and tetracycline (30 µg).

The type strain, BUZ 2^T (=CCUG 58136^T =DSM 22563^T), was isolated from mud from a tidal flat on the North Sea coast of Germany.

Emended description of the genus *Rudanella* Weon *et al.* 2008

The description is as given by Weon *et al.* (2008) with the following emendation. The major respiratory quinone is MK-7, but a small amount of MK-6 is also detected. The antibiotic sensitivity profile of the type species is the same

Fig. 2. Electron micrographs of strain BUZ 2^T. (a) Scanning electron micrograph showing filamentous cells. (b) Transmission electron micrograph of a longitudinal section and cross-sections of bacterial cells. Bars, 1 µm.

as that of *Fibrella aestuarina*, with the exception that *Rudanella lutea* is sensitive to vancomycin. The genus is a member of the family *Cytophagaceae*, phylum *Bacteroidetes*.

Acknowledgements

This work was supported by the Faculty of Science of the University of Zurich. We thank K. Finster, C. N. Seong, W.-T. Im and S.-W. Kwon for kindly providing submitted papers on the characterization of *Spirosoma spitsbergense*, *S. luteum*, *S. panaciterrae*, *S. rigui* and *Rudanella lutea*, M. Laternus for help during sampling, G. Barmettler and K. Marquardt for performing electron microscopy and M. Eisenring for help during phenotypic tests. We thank the editor for extensive comments, in addition to two anonymous reviewers who helped to improve the manuscript.

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