Friedmanniella luteola sp. nov., *Friedmanniella lucida* sp. nov., *Friedmanniella okinawensis* sp. nov. and *Friedmanniella sagamiharensis* sp. nov., isolated from spiders

Kimika Iwai, Kazuo Aisaka and Makoto Suzuki†

Correspondence

Kimika Iwai

kimika.iwai@kyowa-kirin.co.jp

Innovative Drug Research Laboratories, Kyowa Hakko Kirin Co., Ltd 3-6-6, Asahi-machi, Machida-shi, Tokyo 194-8533, Japan

Four Gram-positive, non-motile, aerobic actinobacteria were isolated from spiders and their webs. Their genetic, phenotypic and chemical properties were studied. The 16S rRNA gene sequence data suggested that the four novel isolates belonged to the genus *Friedmanniella*. Two strains (FA1^T and FA2^T) formed a cluster together with *Friedmanniella capsulata* and *Friedmanniella lacustris* and the other two strains (FB1^T and FB2^T) formed a cluster together with *Friedmanniella antarctica* and *Friedmanniella spumicola*. The cell-wall peptidoglycan contained LL-A₂pm and mycolic acids were absent. Isoprenoid quinones were mainly composed of MK-9(H4), MK-9(H2) and MK-9 and the predominant fatty acids were 12-methyltetradecanoic acid (ai-C_{15:0}) and 13-methyltetradecanoic acid (i-C_{15:0}). The major polar lipids were phosphatidylinositol and phosphatidylglycerol. In addition, strain FA1^T, FB1^T, and FB2^T contained diphosphatidylglycerol and phosphatidylcholine. The DNA G+C contents were: 72 mol%, 73 mol%, 74 mol% and 75 mol% for strains FA1^T, FA2^T, FB1^T, and FB2^T, respectively. DNA–DNA hybridization studies demonstrated that the novel strains showed low relatedness values to *F. capsulata*, *F. lacustris*, *F. antarctica* and *F. spumicola*. These data support the proposal that strains FA1^T, FA2^T, FB1^T and FB2^T represent novel species of the genus *Friedmanniella*. Therefore, the names *Friedmanniella luteola* (type strain FA1^T=DSM 21741^T=NBRC 104963^T), *Friedmanniella lucida* (type strain FA2^T=DSM 21742^T=NBRC 104964^T), *Friedmanniella okinawensis* (type strain FB1^T=DSM 21744^T=NBRC 104966^T) and *Friedmanniella sagamiharensis* (type strain FB2^T=DSM 21743^T=NBRC 104965^T) are proposed for these new strains.

During a search for new secondary metabolites, actinobacteria other than the genus *Streptomyces* were investigated as potential producing strains. Spiders and their webs were chosen as an alternative to soil samples as they are easy to collect and have not thus far been used for microbe isolation. Isolates typical of the genus *Friedmanniella* were obtained at high frequency from spiders and four strains were selected for further analyses.

The genus *Friedmanniella*, containing LL-diaminopimelic acid (LL-A₂pm) as the diagnostic cell-wall diamino acid, was first established by Schumann *et al.* (1997) as a member of the family *Nocardioideae*. At present, the genus contains four recognized species. *Friedmanniella antarctica* was isolated from Antarctic sandstone (Schumann *et al.*, 1997), *Friedmanniella spumicola* and *Friedmanniella capsulata* were isolated from activated sludge samples (Maszenan *et al.*, 1999) and *Friedmanniella lacustris* was reported from a hypersaline Antarctic lake (Lawson *et al.*, 2000). In this paper, four novel actinobacterial isolates from spiders are described as novel species of the genus *Friedmanniella*.

All spider samples were collected in a Japanese forest. Spider webs were placed on HV agar [in a litre distilled water: 1.0 g humic acid, 0.5 g Na₂HPO₄, 1.71 g KCl, 0.05 g MgSO₄·7H₂O, 0.01 g FeSO₄·7H₂O, 0.02 g CaCO₃, 1.0 ml B vitamin mixture (50 mg thiamin-HCl, 50 mg riboflavin, 50 mg niacin, 50 mg pyridoxin-HCl, 50 mg inositol, 50 mg calcium pantothenate, 50 mg *p*-aminobenzoic acid,

†Present address: Kyowa Hakko Bio Co., Ltd, 1-6-1 Ohtemachi, Chiyoda-ku, Tokyo 100-8185, Japan.

Abbreviations: 2D TLC, two-dimensional thin layer chromatography, LL-A₂pm, LL-diaminopimelic acid; SEM, scanning electron microscopy.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains FA1^T (=A34_1^T), FA2^T (=M2_8^T), FB1^T (=O4_4^T) and FB2^T (=S20_27^T) are AB445453–AB445456, respectively.

A supplementary table detailing the polar lipid properties for the spider isolates is available with the online version of this paper.

25 mg biotin in 100 ml distilled water), 18.0 g agar; pH 7.2] (Hayakawa & Nonomura, 1987; Hayakawa *et al.*, 2000) with antibiotics (cycloheximide 100 mg l⁻¹, nystatin 50 mg l⁻¹, nalidixic acid 10 mg l⁻¹ and trimethoprim 20 mg l⁻¹). Alternatively, a spider was allowed to walk over an agar plate. The plate surface was then sprayed twice with 2 ml 70 % ethanol to inhibit the growth of other bacteria and fungi. After spraying, the spider web was dissolved and the remaining material was spread until dry. The plates were incubated aerobically at 28 °C for 3 weeks. Culture purification and cell biomass preparation were conducted using KM398 medium (5 g glucose, 5 g soluble starch, 1.5 g beef extract, 2.5 g yeast extract, 2.5 g tryptone peptone (Difco), 0.5 g MgSO₄·7H₂O, 1 g KH₂PO₄, 0.5 g Mg₃(PO₄)₂·8H₂O, 1 l distilled water, pH 7.2) at 25 °C for 14 days and the biomass was used for chemotaxonomic studies. The isolates were preserved in 20 % glycerol solution at -80 °C until use. Strain FA1^T was isolated from the surface of the body of a specimen of the spider *Diaea subdola* collected in Yamanashi prefecture, Japan. Strains FA2^T, FB1^T and FB2^T were isolated from spiders' silk or web. Strain FA2^T was isolated from *Agelena silvatica* (Tokyo), strain FB1^T was from unidentified spider (Okinawa) and strain FB2^T was from *Agelena silvatica* (Kanagawa). The strains used for comparison were *F. antarctica* DSM 11053^T, *F. lacustris* DSM 11465^T and *F. capsulata* DSM 12936^T. Due to problems in the acquisition of *F. spumicola* ACM 5121^T, only previously published data were used for comparisons for this species.

PCR amplification of the 16S rRNA gene of the isolates from spiders was carried out following the methods of Tanner *et al.* (2000). The amplified fragments were purified by a MagExtractor kit (Toyobo). Sequencing was carried out with a BigDye Terminator Cycle Sequencing FS Ready Reaction kit and an ABI 377 DNA sequencer (Applied Biosystems). The partial sequences (1420 bp) of the 16S rRNA gene were aligned using MEGA4.0 software (Tamura *et al.*, 2007). Genomic DNA preparation, DNA G+C content determination and DNA-DNA hybridization experiments were conducted according to Suzuki *et al.* (1999, 2001).

For scanning electron microscopy (SEM) observations, the strains were cultured on 1/2 HV (containing half the usual amount of humic acid) agar for 14–21 days at 25 °C. An agar block containing strain growth was fixed with osmium tetroxide vapour, washed with distilled water, dehydrated through a graded ethanol series, transferred into amylacetate and finally critical-point dried with liquid carbon dioxide (HCP-2; Hitachi). The specimens were sputter-coated with platinum-palladium (E-102; Hitachi) and observed via scanning electron microscopy (S-570LB; Hitachi).

Cell-wall diamino acids were analysed as described by Stanek & Roberts (1974) and cell wall acyl type was determined as described by Uchida *et al.* (1999). Purified cell walls were obtained using the method of Kawamoto

et al. (1981), and the amino acid composition of hydrolysed cell walls was determined using cellulose TLC with n-butanol/acetic acid/H₂O (4:1:2) as the development system (Matsumoto *et al.*, 2007). Absence of mycolic acids was demonstrated following Yano *et al.* (1972). Cellular fatty acid profiles were determined as described by Miller (1982) with a GC-2010 (Shimadzu) and the authentic standards (Bacterial Acid Methyl Esters CP Mix; Matreya Inc.). Menaquinones were extracted following Collins *et al.* (1977) and the molecular species were determined by HPLC (Tamaoka *et al.*, 1983). Polar lipids were extracted according to the method of Minnikin *et al.* (1984) and analysed by MS as described by Taguchi *et al.* (2000). Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS) was used to perform the assignment of the head group by the position sensitive detector (PSD) measurement. Molecular species and the length of the acyl chain were identified from the retention time and mass spectrum with LC/ESI (electro spray ionization).

All physiological tests were performed at 25 °C unless otherwise stated. The cell suspensions for inoculation were prepared with 10 day cultures on KM398 agar at 25 °C. Growth at different temperatures (between 6–37 °C), NaCl concentrations (0–10 % at intervals of 2 %, w/v) and pH (5.0–9.0 at intervals of 0.5 pH unit) were determined on KM398 agar or broth. Carbon source utilization was determined using ISP 9 medium (Pridham & Gottlieb, 1948) supplemented with 0.05 % yeast extract, 1 ml l⁻¹ of B vitamins (Hayakawa *et al.*, 2000) and 1 % (w/v) of each substrate. Aerobic reduction of nitrate to nitrite or N₂, urease activity, protease activity, production of indole and acid production from glucose were assessed by API 20NE systems (bioMérieux). Catalase production was evaluated by the evolution of bubbles from 3 % H₂O₂. Cytochrome oxidase activities were tested with cytochrome oxidase reagent (Nissui Pharmaceutical). Production of hydrogen sulfide from peptone was evaluated following the method of Tresner & Danga (1958). Starch hydrolysis was tested by observing the appearance of clear zones on inorganic salts-starch agar (ISP 4), following Goodfellow & Pirouz (1982). The biochemical characteristics of all strains were determined using API 20NE kits (bioMérieux).

Samples derived from spiders usually contain many kinds of fast-growing bacteria and fungi and the pre-treatment method using 70 % ethanol effectively prevented their growth. Members of the genus *Friedmanniella* and other actinobacteria seemed to show tolerance to 70 % ethanol since diverse actinobacteria (including members of the genus *Friedmanniella*) were observed after this treatment. A total of 1159 actinobacterial strains were obtained from 244 spider samples and the strains included representatives from at least 29 genera (Iwai *et al.*, 2009). Members of the suborder *Propionibacterineae*, such as the genera *Friedmanniella* and *Microtholunatus*, appeared to be abundant in the samples from spiders as compared to isolates obtained from soil samples. The percentage of representa-

tives of the genus *Friedmanniella* in the 1159 actinobacterial strains was about 2 %. *Friedmanniella*-like strains were acquired from funnel-webs, orb webs, tangle-webs and crab spiders (non-web-producers) with high frequency. This study focused on members of the genus *Friedmanniella* and the four unique strains that were identified. The species of the genus *Friedmanniella* described previously have been isolated from so-called harsh environments, but we could effectively isolate them from spiders and their webs after pretreatment with 70 % ethanol. A possible reason why species of the genus *Friedmanniella* have not been isolated previously from ordinary soil samples could be due to competition with other soil micro-organisms, such as members of the genus *Streptomyces*. The relative percentage of *Friedmanniella* species in soil is very low and it is very difficult to isolate them by a direct plating method. Members of the genus *Friedmanniella* are probably common in a wide range of environments. The reasons why species of the genus *Friedmanniella* have been isolated in this study could be that spider environments are particularly suitable for such species or because the pretreatment with 70 % ethanol could inhibit the growth of other micro-organisms more than that of members of the genus *Friedmanniella*.

Phylogenetic analysis of the 16S rRNA gene sequences (1441 bp) of strains FA1^T, FA2^T, FB1^T and FB2^T revealed

that the four strains formed a tight cluster with species of the genus *Friedmanniella* and were supported by a high bootstrap value of 100 % (Fig. 1). Strains FA1^T and FA2^T were most closely related to *F. capsulata* and *F. lacustris*, with sequence similarity levels of >97 %, whereas strains FB1^T and FB2^T were most closely related to *F. antarctica* and *F. spumicola*, with sequence similarity levels of >98 %. Strains FA1^T and FA2^T, *F. capsulata* and *F. lacustris* were therefore classified as *Friedmanniella* group A, whereas strains FB1^T and FB2^T, *F. antarctica* and *F. spumicola* were classified as *Friedmanniella* group B, as shown in Fig. 1. These phylogenetic results strongly suggested that the isolates belong to the genus *Friedmanniella*.

All isolates were chrome yellow to orange in colour, non-motile, slow-growing, Gram-positive and grew as clusters of coccoid cells, in which bunches of 2 or 4 cells were observed under the microscope (Fig. 2). Strains FA2^T, FB1^T and FB2^T produced extracellular polymers after prolonged cultivation. Strain FA1^T did not produce extracellular polymers and had crumbly colonies.

The cell-wall peptidoglycan of all four isolates contained LL-A₂pm as the diagnostic diamino acid and the interpeptide bridges had a single glycine residue, at position 1. These results were consistent with the type A3 γ peptidoglycan, which is found in the genus *Friedmanniella* (Schleifer

Fig. 1. Phylogenetic tree of the genus *Friedmanniella* and related actinomycetes based on 16S rRNA gene sequences, constructed using the neighbour-joining method (Saitou & Nei, 1987). *Streptomyces griseus* ATCC 10137^T (GenBank accession no. Y15501) was used as the outgroup. Filled circles indicate generic branches recovered with the maximum-parsimony algorithm. Branches corresponding to partitions reproduced in <60 % bootstrap replicates are collapsed. The number at each node indicates the percentage bootstrap value of 1000 replicates (Felsenstein, 1985). Bar, genetic distance of 0.01 (K_{MUC}). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in units of number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). There were a total of 1441 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura *et al.*, 2007).

Fig. 2. SEM images of cells of strains FA1^T, FA2^T, FB1^T and FB2^T isolated from spiders. (a) Strain FA1^T showing a colony of aggregated cells; (b) strain FA2^T; (c) strain FB1^T; (d) strain FB2^T showing regular packages associated into aggregates. Bars, (a) 2 µm; (b, c) 5 µm; (d) 1 µm.

& Seidl, 1985). Mycolic acids were not detected. The cellular fatty acid profile of strain FA1^T was characterized by the predominance of ai-C_{15:0} (12-methyltetradecanoic acid; 71.3 %), i-C_{15:0} (13-methyltetradecanoic acid; 26.8 %) and trace amounts of C_{16:0} (methylhexadecanoic acid), i-C_{16:0} (14-methylpentadecanoic acid) and i-C_{17:0} (15-methylhexadecanoic acid). Strains FA2^T and FB1^T

showed similar fatty acid contents to strain FA1^T. Strain FA2^T contained ai-C_{15:0} (60.6 %) and i-C_{15:0} (37.1 %) and strain FB1^T had ai-C_{15:0} (59.3 %) and i-C_{15:0} (37.5 %) as the dominant fatty acids. On the other hand, strain FB2^T was mainly composed of i-C_{15:0} (59.1 %) and ai-C_{15:0} (37.4 %). Additional differences between the fatty acid contents of the four novel strains are shown in Table 1. Since the results of fatty acid analyses vary to some degree between laboratories and the methods used also differ to some extent, the fatty acid profiles of the type strains of the genus *Friedmanniella* were all determined under standardized conditions. Isoprenoid quinones were mainly composed of MK-9(H₄), MK-9(H₂) and MK-9. Strains FA1^T and FB1^T had all three quinones at ratios of 67:24:9 and 74:21:5, respectively. Strain FB2^T had MK-9(H₄) and MK-9(H₂) at a ratio of 77:23. Strain FA2^T possessed only MK-9(H₄). The four novel strains contained phosphatidylinositol and phosphatidylglycerol as the polar lipids. In addition, strains FA1^T, FB1^T and FB2^T also contained diphosphatidylglycerol and phosphatidylcholine. The existence of a previously unidentified phosphatidylcholine was demonstrated using MS for analysis of the polar lipids (see Supplementary Table S1 in IJSEM Online). The DNA G + C contents were 72 mol% for strain FA1^T, 73 mol% for strain FA2^T, 74 mol% for strain FB1^T and 75 mol% for strain FB2^T.

The results of the DNA–DNA hybridization analysis were as follows. In group A, strains FA1^T and FA2^T showed only 29 % and 43 % relatedness to *F. lacustris* and *F. capsulata*, respectively. Strains FA1^T and FA2^T showed 46 % relatedness to each other. In group B, strains FB1^T and FB2^T both showed 15 % DNA–DNA relatedness to *F. antarctica*. Strains FB1^T and FB2^T showed 11 % and 30 % relatedness, respectively, to *F. spumicola*. Strains FB1^T and FB2^T showed only 19 % relatedness to each other. These values were below the 70 % cut-off point for species delineation recommended by Wayne *et al.* (1987). In addition, characteristics such as the mode of cell aggregation, DNA G + C contents and presence

Table 1. Cellular fatty acids contents of isolates FA1^T, FA2^T, FB1^T and FB2^T and recognized species of the genus *Friedmanniella*

Strains: 1, FA1^T; 2, FA2^T; 3, *F. lacustris* DSM 11465^T; 4, *F. capsulata* DSM 12936^T; 5, FB1^T; 6, FB2^T; 7, *F. spumicola* ACM 5121^T; 8, *F. antarctica* DSM 11053^T. All data from this study except for taxon 7, which are from Maszenan *et al.* (1999).

Fatty acid	Group A				Group B			
	1	2	3	4	5	6	7	8
i-C _{14:0}								3.9
C _{14:0}					1.1	0.5		3.3
i-C _{15:0}	26.8	37.1	14.1	18.3	37.5	59.1	15.1	13.3
ai-C _{15:0}	71.3	60.6	83.8	71.4	59.3	37.4	81.8	57.7
C _{15:0}			2.1				2.5	3.2
i-C _{16:0}	0.4	1.7		6.3		0.2	0.6	0.0
C _{16:1} ⁹						0.5		0.0
C _{16:0}	1.1	0.3		1.2	1.8	1.1		4.5
i-C _{17:0}	0.4	0.2		2.8	0.4	0.4		
C _{18:1}						0.9		14.1

of phosphatidylcholine (except for strain FA2^T) suggested that the four strains isolated from spiders were novel species of the genus *Friedmanniella*.

All isolates grew optimally at 25 °C and at pH 7.0. Strain FA1^T was different from *F. lacustris* and *F. capsulata* as regards the utilization of raffinose and inositol and lack of utilization of xylose. Strains FB1^T and FB2^T differed from *F. antarctica* and *F. spumicola* in their utilization of rhamnose, sucrose, raffinose and inositol and did not produce hydrogen sulfide. DNA–DNA hybridization studies of *F.*

spumicola with strains FB1^T and FB2^T are not described in this study. However, carbon source utilization data for strains FB1^T and FB2^T supported their differentiation from *F. spumicola*. Urease activity and the hydrolysis of gelatin were positive for all isolates. Catalase activity was positive for strains FA1^T, FA2^T and FB1^T, but was negative for strain FB2^T. Reduction of nitrate to nitrite, production of hydrogen sulfide and indole, oxidase activity, hydrolysis of starch and acid production from glucose were also negative for all strains. Other physiological and biochemical properties are listed in Table 2 and in the species

Table 2. Characteristics of isolates FA1^T, FA2^T, FB1^T, FB2^T (group A, B) compared with related species of the genus *Friedmanniella*

Strains: 1, FA1^T; 2, FA2^T; 3, *F. lacustris* DSM 11465^T (data from Lawson *et al.*, 2000); 4, *F. capsulata* DSM 12936^T (Maszenan *et al.*, 1999); 5, FB1^T; 6, FB2^T; 7, *Friedmanniella spumicola* ACM 5121^T (Maszenan *et al.*, 1999); 8, *F. antarctica* DSM 11053^T (Schumann *et al.*, 1997). +, Positive; –, negative; w, weakly positive.

Characteristics	Group A				Group B			
	1	2	3	4	5	6	7	8
Source	Surface of spider body	Spider web	Antarctic hypersaline Ekho Lake	Sewage treatment plant	Spider web	Spider web	Sewage treatment plant	Antarctic sandstone
Cell diameter (µm)	1.1–1.6	0.5–0.8	1.0–1.3	0.6–1.2	0.6–0.7	0.7–1.0	0.5–1.4	0.5–2.2
Production of extracellular polymer	–	+	+	+	+	+	+	–
Temperature (°C)								
Range for growth	18–28	18–32	3–43.5	15–30	18–32	18–32	15–37	9–25
Optimum	25–27	22–28	26	20–25	22–28	25–28	25	22
pH								
Range for growth	5.3–8.5	5.0–9.0	5.5–9.5	5.5–7.5	5.0–9.0	5.0–9.0	5.5–8.0	5.1–8.1
Optimum	6.0–7.7	6.0–7.0	7.5	6.5–7.0	6.0–7.0	7.0–7.7	7.0–7.5	6.0–7.2
Growth range for NaCl (% w/v)	0–2	0–2	0–(4)–6–8	Inhibition	0–4	0–6	Inhibition	0–2
Carbon sources utilized†								
L-Arabinose	w	–	+	+	+	+	+	w
D-Fructose	w	–	+	+	+	+	+	–
D-Galactose	w	w	+	–	+	+	+	–
D-Glucose	–	+	+	–	+	+	+	–
myo-Inositol	w	–	+	–	w	w	–	–
Mannitol	–	+	+	–	+	+	+	–
Raffinose	w	+	+	–	+	+	–	–
L-Rhamnose	–	–	–	+	+	w	–	–
D-Ribose	–	–	–	+	w	+	+	+
Sucrose	–	+	+	+	+	+	–	–
D-Xylose	–	–	+	+	+	+	+	w
Polymer hydrolysis:								
Gelatin*	+	+	+	–	+	+	+	–
Starch*	–	–	+	–	–	–	–	+
Acid produced from:								
D-Glucose*	–	–	+	–†	–	–	–†	–
Catalase*	+	+	+	+	+	–	+	+
Oxidase*	–	–	w	–	–	–	–	–
Urease*	+	+	–	+	+	+	+	+
Production of H ₂ S/indole*	–/–	–/–	–/–	+/–	–/–	–/–	+/–	+/–
NO ₃ [–] reduction to NO ₂ [–]	–	–	w	–	–	–	–	–

*Results obtained by API 20NE.

†Results obtained in this study.

descriptions. The characteristics that differentiate the new strains isolated from spiders from the recognized species of the genus *Friedmanniella* are also presented.

In summary, the characteristics of these actinomycetes isolated from spiders were consistent with members of the genus *Friedmanniella* with regards to morphology, biochemistry and chemotaxonomy. However, their phenotypic, biochemical and chemotaxonomic characteristics were different from recognized species of the genus *Friedmanniella* (Tables 1, 2 and Supplementary Table S1). Low DNA–DNA relatedness values were detected between the new isolates and between the isolates and recognized species. From the results of this study, it is apparent that strains FA1^T, FA2^T, FB1^T and FB2^T represent four separate novel species of the genus *Friedmanniella*, for which the names *Friedmanniella luteola* (strain FA1^T), *Friedmanniella lucida* (strain FA2^T), *Friedmanniella okinawensis* (strain FB1^T) and *Friedmanniella sagamiharensis* (strain FB2^T) are proposed.

Description of *Friedmanniella luteola* sp. nov.

Friedmanniella luteola (lu.te'ō.la. L. fem. adj. *luteola* referring to the yellow colour of the colonies).

Coccoid cells are 1.1–1.6 µm in diameter with a smooth surface. The cells are arranged singly or in pairs and adhere to form clusters. The colony is crumbly. Growth in submerged, standing and shaking cultures is poor. Colonies grown aerobically on KM364 agar for 2 weeks are small, creamy yellow, convex and brittle. The temperature range for growth is 18–28 °C and optimum growth temperature is 25–27 °C. Growth occurs at pH 5.3–8.5; the optimum pH range is 6.0–7.7. NaCl tolerance is up to 2 % (w/v); optimum growth occurs with no addition of NaCl. Catalase-positive. Oxidase-negative. Urease-positive. Does not reduce nitrate to nitrite. No acid is produced from D-glucose. Gelatin is hydrolysed, but starch is not hydrolysed. Negative for production of hydrogen sulfide and indole. L-Arabinose, D-galactose, D-fructose, raffinose and myo-inositol are weakly utilized, but D-ribose, D-xylose, D-glucose, L-rhamnose, sucrose and D-mannitol are not utilized. Predominant cellular fatty acids are ai-C_{15:0} and i-C_{15:0} and MK-9(H₄), MK-9(H₂), MK-9 are the major menaquinones. Polar lipids are phosphatidylglycerol, phosphatidylinositol, phosphatidylcholine and diphosphatidylglycerol. Diacylglycerol dihexose is another neutral lipid. Mycolic acids are absent.

The type strain, FA1^T (=DSM 21741^T=NBRC 104963^T), was isolated from the surface of the spider *Diaea subdola* from Yamanashi Prefecture, Japan. The DNA G+C content of the type strain is 72 mol%.

Description of *Friedmanniella lucida* sp. nov.

Friedmanniella lucida (lu'ci.da. L. fem. adj. *lucida* brilliant, shining, referring to the shiny colony surface).

Coccoid cells are 0.5–0.8 µm in diameter with a smooth surface. Cells are arranged in regular packets that adhere to form clusters. Growth in submerged, standing and shaking cultures is poor. Colonies grown aerobically on KM364 agar for 2 weeks are creamy yellow. The colony surface is crumbly, convex, watery and shiny depending on the growth medium. The temperature range for growth is 18–32 °C; optimum growth temperature is 22–28 °C. Growth occurs at pH 5.0–9.0; optimum pH range is 5.0–7.0. NaCl tolerance up to 2 % (w/v), optimum growth occurs with no addition of NaCl. Catalase-positive. Oxidase-negative. Urease-positive. Does not reduce nitrate to nitrite. No acid is produced from D-glucose. Gelatin is hydrolysed, but starch is not hydrolysed. Negative for production of hydrogen sulfide and indole. D-Glucose, D-galactose (weakly), sucrose, raffinose and D-mannitol are utilized. L-Arabinose, D-ribose, D-xylose, D-fructose, L-rhamnose and myo-inositol are not utilized. Predominant cellular fatty acids are ai-C_{15:0}, i-C_{15:0} and i-C_{16:0}. The major menaquinone is MK-9(H₄). Polar lipids are phosphatidylglycerol and phosphatidylinositol. Mycolic acids are absent.

The type strain, FA2^T (=DSM 21742^T=NBRC 104964^T), was isolated from the web of the spider *Agelena silvatica* from Mitake ravine, Tokyo, Japan. The DNA G+C content of the type strain is 73 mol%.

Description of *Friedmanniella okinawensis* sp. nov.

Friedmanniella okinawensis (o.ki.na.wens'is. N.L. fem. adj. *okinawensis* pertaining to Okinawa, a prefecture in Japan, where the organism was first isolated).

Coccoid cells are 0.6–0.7 µm in diameter with a smooth surface. Cells are arranged in regular packets that adhere to form clusters. Growth in submerged, standing and shaking cultures is poor. Colonies grown aerobically on KM364 agar for 2 weeks are orange. The colony surface is convex, watery and spread-out depending on the growth medium. The temperature range for growth is 18–32 °C; the optimum growth temperature is 22–28 °C. Growth occurs at pH 5.0–9.0; optimum pH is 6.0–7.0. NaCl tolerance is up to 4 % (w/v); optimum growth occurs with no addition of NaCl. Catalase-positive. Oxidase-negative. Urease-positive. Does not reduce nitrate to nitrite. No acid is produced from D-glucose. Gelatin is hydrolysed, but starch is not hydrolysed. Negative for production of hydrogen sulfide and indole. L-Arabinose, D-ribose (weakly), D-xylose, D-glucose, D-galactose, D-fructose, L-rhamnose, sucrose, raffinose, myo-inositol (weakly) and D-mannitol are utilized. Predominant cellular fatty acids are: ai-C_{15:0}, i-C_{15:0}, i-C_{16:0} and i-C_{17:0}, and the major menaquinones are MK-9(H₄), MK-9(H₂) and MK-9. Polar lipids are phosphatidylglycerol, phosphatidylinositol, phosphatidylcholine and diphosphatidylglycerol. Mycolic acids are absent.

The type strain, FB1^T (=DSM 21744^T=NBRC 104966^T), was isolated from an unidentified orb web spider from

Okinawa Prefecture, Japan. The DNA G + C content of the typestrain is 74 mol%.

Description of *Friedmanniella sagamiharensis* sp. nov.

Friedmanniella sagamiharensis (sa.ga.mi.ha.ren'sis. N.L. fem. adj. *sagamiharensis* pertaining to Sagamihara, a city in Japan, where the organism was first isolated).

Coccoid cells are 0.7–1.0 µm in diameter with a smooth surface. Cells are arranged in pairs or groups of four and adhere to form clusters. Growth in submerged, standing and shaking cultures is poor. Colonies grown aerobically on KM364 agar for 2 weeks are orange. The colony surface is crumbly and convex to watery (by production of extracellular polymer) and spread out depending on the growth medium. The temperature range for growth is 18–32 °C; the optimum growth temperature is 25–28 °C. Growth occurs at pH 5.0–9.0 and the optimum pH is 7.0–7.7. NaCl tolerance is up to 6% (w/v), optimal growth occurs with no addition of NaCl. Catalase and oxidase-negative. Urease-positive. Does not reduce nitrate to nitrite. No acid is produced from D-glucose. Gelatin is hydrolysed, but starch is not hydrolysed. Negative for production of hydrogen sulfide and indole. L-Arabinose, D-ribose, D-xylose, D-glucose, D-galactose, D-fructose, L-rhamnose (weakly), sucrose, raffinose, *myo*-inositol (weakly) and D-mannitol are utilized. Predominant cellular fatty acids are i-C_{15:0} and ai-C_{15:0}, and the major menaquinones are MK-9(H₄) and MK-9(H₂). Polar lipids are: phosphatidylglycerol, phosphatidylinositol, phosphatidylcholine and diphosphatidylglycerol. Diacylglycerol dihexose is another neutral lipid. Mycolic acids are absent.

The type strain, FB2^T (=DSM 21743^T=NBRC 104965^T), was isolated from the web of the spider *Agelena silvatica* collected from Kanagawa Prefecture, Japan. The DNA G + C content of the type strain is 75 mol%.

Acknowledgements

We would like to thank the Tokyo Spider Study Group and the Arachnological Society of Japan. We would particularly like to thank Akira Shinkai and Akio Tanikawa for spider identification.

References

- Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Goodfellow, M. & Pirouz, T. (1982). Numerical classification of sporoactinomycetes containing *meso*-diaminopimelic acid in the cell wall. *J Gen Microbiol* **128**, 503–527.
- Hayakawa, M. & Nonomura, H. (1987). Humic acid-vitamin agar, a new medium for selective isolation of soil actinomycetes. *J Ferment Technol* **65**, 501–509.
- Hayakawa, M., Ootoguro, M., Takeuchi, T., Yamazaki, T. & Iimura, Y. (2000). Application of a method incorporating differential centrifugation for selective isolation of motile actinomycetes in soil and plant litter. *Antonie van Leeuwenhoek* **78**, 171–185.
- Iwai, K., Iwamoto, S., Aisaka, K. & Suzuki, M. (2009). Isolation of novel actinomycetes from spider materials. *Actinomycetologica* **23**, 8–15.
- Kawamoto, I., Oka, T. & Nara, T. (1981). Cell wall composition of *Micromonospora olivoasterospora*, *Micromonospora sagamiensis*, and related organisms. *J Bacteriol* **146**, 527–534.
- Lawson, P. A., Collins, M. D., Schumann, P., Tindall, B. J., Hirsch, P. & Labrenz, M. (2000). New LL-diaminopimelic acid-containing actinomycetes from hypersaline, heliothermal and meromictic Antarctic Ekho lake: *Nocardioides aquaticus* sp. nov. and *Friedmanniella lacustris* sp. nov. *Syst Appl Microbiol* **23**, 219–229.
- Maszenan, A. M., Sevier, R. J., Patel, B. K. C. & Schumann, P. (1999). *Friedmanniella spumicola* sp. nov. and *Friedmanniella capsulata* sp. nov. from activated sludge foam: gram-positive cocci that grow in aggregates of repeating groups of cocci. *Int J Syst Bacteriol* **49**, 1667–1680.
- Matsumoto, A., Takahashi, Y., Fukumoto, M. & Omura, S. (2007). *Actinocatenispora sera* sp. nov., isolated by long-term culturing. *Int J Syst Evol Microbiol* **57**, 2651–2654.
- Miller, L. T. (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584–586.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Pridham, T. G. & Gottlieb, D. (1948). The utilization of carbon compounds by some *Actinomycetales* as an aid for species determination. *J Bacteriol* **56**, 107–114.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Schleifer, K. H. & Seidl, P. H. (1985). Chemical composition and structure of murein. In *Chemical Methods in Bacterial Systematics*, pp. 201–219. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Schumann, P., Prauser, H., Rainey, F. A., Stackebrandt, E. & Hirsch, P. (1997). *Friedmanniella antarctica* gen. nov., sp. nov., an LL-diaminopimelic acid containing actinomycete from Antarctic sandstone. *Int J Syst Bacteriol* **47**, 278–283.
- Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.
- Suzuki, M., Nakagawa, Y., Harayama, S. & Yamamoto, S. (1999). Phylogenetic analysis of genus *Marinilabilia* and related bacteria based on the amino acid sequences of GyrB and emended description of *Marinilabilia salmonicolor* with *Marinilabilia agarovorans* as its subjective synonym. *Int J Syst Bacteriol* **49**, 1551–1557.
- Suzuki, M., Nakagawa, Y., Harayama, S. & Yamamoto, S. (2001). Phylogenetic analysis and taxonomic study of marine cytophaga-like bacteria: proposal for *Tenacibaculum* gen. nov. with *Tenacibaculum maritimum* comb. nov. and *Tenacibaculum ovolyticum* comb. nov., and *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amyolyticum* sp. nov. *Int J Syst Evol Microbiol* **51**, 1639–1652.
- Taguchi, R., Hayakawa, J., Takeuchi, Y. & Ishida, M. (2000). Two-dimensional analysis of phospholipids by capillary liquid chromatography/electrospray ionization mass spectrometry. *J Mass Spectrom* **35**, 953–966.
- Tamaoka, J., Katayama-Fujimura, Y. & Kuraishi, H. (1983). Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. *J Appl Bacteriol* **54**, 31–36.

Tamura, K., Nei, M. & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc Natl Acad Sci U S A* **101**, 11030–11035.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596.

Tanner, M. A., Everett, C. L. & Youvan, D. C. (2000). Molecular phylogenetic evidence for noninvasive zoonotic transmission of *Staphylococcus intermedius* from a canine pet to a human. *J Clin Microbiol* **38**, 1628–1631.

Tresner, H. D. & Danga, F. (1958). Hydrogen sulfate production by *Streptomyces* as a criterion for species differentiation. *J Bacteriol* **76**, 239–244.

Uchida, K., Kudo, T., Suzuki, K. & Nakase, T. (1999). A new rapid method of glycolate test by diethyl ether extraction, which is applicable to a small amount of bacterial cells of less than one milligram. *J Gen Appl Microbiol* **45**, 49–56.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Yano, I., Saito, K., Fukukawa, Y. & Kusunose, M. (1972). Structural analysis of molecular species of nocardomycolic acids from *Nocardia erythropolis* by the combined system of gas chromatography and mass spectrometry. *FEBS Lett* **21**, 215–219.