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Flavisolibacter swuensis sp. nov. Isolated from Soil[§]

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A Gram-staining-negative, non-motile, non-spore-forming, and rod-shaped bacterium designated as strain SR2-4-2^T was isolated from soil in South Korea. Phylogenetic analysis based on 16S rRNA gene sequence of strain SR2-4-2^T revealed that it belonged to the genus of *Flavisolibacter*, family of *Chitinophagaceae*, and class of *Sphingobacteriia*. It shared sequence similarities with *Flavisolibacter ginsengisoli* Gsoil 643^T (96.4%), *Flavisolibacter ginsengiterrae* Gsoil 492^T (96.3%), and *Flavisolibacter rigui* 02SUJ3^T (93.0%). Chemotaxonomic data revealed that its predominant fatty acids were iso-$C_{15:0}$ (26.4%) and iso-$C_{17:0}$ 3OH (10.7%). Its major polar lipid was phosphatidylethanolamine (PE) and its predominant respiratory quinone was MK-7. The G+C content of genomic DNA of the strain SR2-4-2^T DNA was 45.0%. Based on the phylogenetic, chemotaxonomic, and phenotypic data, the strain SR2-4-2^T (=JCM 19974^T =KEMB 9004-156^T) is classified as a type strain of a novel species for which the name of *Flavisolibacter swuensis* sp. nov. is proposed.

Keywords: *Flavisolibacter*, *Chitinophagaceae*, taxonomy

Introduction

The genus *Flavisolibacter*, was first prosed by Yoon and Im (2007) and allocated to the family of *Chitinophagaceae* (Kamper *et al*., 2011). At the time of writing, the genus *Flavisolibacter* comprises three species with validly published names (www.bacterio.net/flavisolibacter.html). Members of the genus *Flavisolibacter* are aerobic, Gram-staining-positive, non-spore-forming, yellow pigmented, and non-motile rods. Ionizing radiation resistance in prokaryotic and eukaryotic cells have been reported (Daly, 2009; Ignacio *et al*., 2013; Kang *et al*., 2013) with reduced reactive oxygen species and lower oxidation of key proteins involved in cellular integrity and function. DNA damage due to ionizing radian could be reversed by nucleotide excision repair pathway (Kim *et al*., 2015) to protect damaged DNA and cell.

During a study to isolate radiation-resistant bacterial strains from soils, we isolated a strain (designated SR2-4-2^T) from a gamma ray-irradiated soil collected at Seoul Women's University (GPS; N 37° 37′ 41″ E 127° 5′ 25″), South Korea. Results of polyphasic taxonomic investigation indicated that the strain SR2-4-2^T represented a novel *Flavisolibacter* species.

Materials and Methods

Isolation of bacterial strain and culture conditions

The bacterial strain SR2-4-2^T was isolated from a soil sample (pH 6.6) collected at Seoul Women's University, Seoul, South Korea. The soil sample was exposed to gamma radiation (5 kGy using a cobal-60 gamma irradiator; point source; AECL, IR-79), which eliminate the susceptible microorganisms in the soil. One gram of the irradiated soil was immersed in 10 ml of saline (0.85% w/v NaCl) followed by serial dilution. A total of 100 µl of each dilution was spread onto a 1/2 R2A agar plate (Difco) and incubated at 30°C. The colonies on the agar plate were purified by sub-culturing onto new R2A agar plates. Purified colony was identified by partial 16S rRNA gene sequence using EzTaxon Sever (http://eztaxon-e.ezbiocloud.net) (Kim *et al*., 2012).

The isolated strain SR2-4-2^T was deposited at Japan Collection of Microorganisms (JCM 19974^T) and Korea Environmental Microorganisms Bank (KEMB 9004-156^T). Type strains *Flavisolibacter gensengisoli* KCTC 12657^T, *Flavisolibacter ginsengiterrae* KCTC 12656^T, and *Flavisolibacter rigui* KCTC 23328^T were obtained from the Korean Collection for Type Cultures (KCTC). All strains were cultivated and maintained on R2A agar plates (Difco) unless otherwise stated.

16S rRNA sequencing and phylogenetic analysis

The 16S rRNA gene of the strain SR2-4-2^T was amplified using 9F and 1492R universal bacterial primers (Weisburg *et al*., 1991) and sequenced by Genotech using 9F, 518F, 785F, and 800R universal primers. Sequences of the 16S rRNA genes were analyzed with SeqMan software (DNASTAT Inc.). Sequences were compared to each other using the EzTaxon-e server and NCBI Blast program. The 16S rRNA sequences of related taxa were obtained from GenBank and edited with BioEdit program (Hall, 1999). Multiple sequence alignments were performed with CLUSTAL_X program (Thompson *et*

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The NCBI GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SR2-4-2^T (=JCM 19974^T =KEMB 9004-156^T) is KJ461520.

al., 1997). Phylogenetic tree was constructed using MEGA5 program (Tamura *et al*., 2011). The phylogenetic tree was constructed using neighbor-joining (NJ) algorithm (Saitou and Nei, 1987) with pairwise distances calculation according to the Kimura two-parameter model (Kimura, 1983). Bootstrap analysis (Felsenstein, 1985) was performed with 1,000 replicates. Min-mini heuristic method (Fitch, 1971) with a search factor of one was applied in a maximum-parsimony (MP) analysis (MEGA 5 Program).

Phenotypic and biochemical characteristics

Cell morphology was examined using a light microscope (Nikon E600) and transmission electron microscopy (Call Zeiss LEO912AB). A classical Gram-staining reaction was performed as described by Gerhardt *et al*. (1994). Motility was determined using the hanging drop technique after the cells had grown for 2 days at 30°C on R2A agar plate. Anaerobic growth was determined on R2A agar plates using GasPak jars (BBL) at 30°C. Growth on different media was examined using R2A agar plates (Difco), nutrient agar (NA, Difco), and trypticase soy agar (TSA, Difco). Oxidase activity was evaluated using 1% (w/v) tetramethyl-p-phenylene diamine. Catalase activity was determined by applying 3% (v/v) hydrogen peroxide solution. Carbon source utilization and production of enzyme were determined using API ZYM and API 20NE microtest systems according to the recommendations of the manufacturer (bioMérieux). The hydrolysis of casein, starch, Tween 80, aesculin, chitin, DNA, gelatin, and xylan were tested on R2A agar plates according to methods described previously (Lanyi, 1987; Smibert and Krieg, 1994). Growth at various temperatures (10, 15, 20, 25, 30, 35, 37, and 42°C) were examined on R2A agar plates for 3 days. Growth at various pH levels (5, 6, 7, 8, 9, and 10) were assessed in R2A broth (MBcell) at 30°C. The pH of the medium was maintained using three buffers (final concentration of 50 mM): acetate buffer (for pH 4.0–5.0), phosphate buffer (for pH 6.0–8.0), and Tris buffer (for pH 9.0–10.0). Tolerance to NaCl was analyzed on R2A broth (MBcell) amended with 0–5% (w/v) NaCl (0.5% intervals) at 30°C.

Gamma radiation-resistance analysis

The survival rate of strain SR2-4-2^T after exposure to gamma radiation was measured and compared with a positive control (*Deinococcus radiodurans* R1^T; DSM 20539^T) and a negative control (*Escherichia coli* K12; KCTC 1116). The early stationary phase (~10^9 CFU/ml) cells were irradiated with a cobalt-60 based gamma irradiator and the irradiation strength was approximately 100 kCi (3.7 PBq) at a dose rate of 70 Gy/min. The irradiated cells were diluted and placed on TGY agar plates. The colony-forming units (CFUs) determined; the survival rates were calculated as described previously (Im *et al*., 2008; Lim *et al*., 2006, 2012; Srinivasan *et al*., 2012a, 2012b, 2014; Lee *et al*., 2014).

Chemotaxonomic and genomic analyses

For fatty acid analysis, cells of strains SR2-4-2^T, KCTC 12657^T, KCTC 12656^T, and KCTC 23328^T were grown on R2A agar plates for 2 days at 30°C. Two loops of well-grown cells at the third and fourth quatrain streaks were harvested. Fatty acid methyl ester profiling was performed using MIDI (Sherlock version 6.01; database TSBA6; MIDI, Inc.) (Sasser, 1990). Major isoprenoid quinones of SR2-4-2^T were extracted, purified via thin-layer chromatography (TLC), and subsequently analyzed by HPLC as described previously (Collins and Jones, 1981; Shin *et al*., 1996). Polar lipids of SR2-4-2^T were extracted using method of Minnikin *et al*. (1984) and identified using two-dimensional thin-layer chromatography (TLC) as described previously (Minnikin *et al*., 1984; Komagata and Suzuki, 1987). Each type of polar lipid spot was detected with appropriate detection reagents as previously described (Lee *et al*., 2013). DNA G+C content was determined using purified genomic DNA (Genomic-tip system 100/G, Qiagen). Genomic DNA was degraded enzymatically into nucleosides and analyzed using reverse-phase high performance liquid chromatography (HPLC) (Tamaoka and Komagata, 1984; Mesbah *et al*., 1989).

Results and Discussion

Morphological and phenotypic characteristics

Cells of SR2-4-2^T were found to be Gram-staining-negative, aerobic, non-motile, non-endospore forming, and rod-shaped (Fig. 1). Colonies on R2A agar were circular with entire margins, opaque, pale yellow, and measured at approximately 2.5 mm in diameter after growing 3 days at 30°C. The growth was observed at 10–30°C (with optimum temperature of 30°C) and pH 7–8 (optimum pH of 7). Strain SR2-4-2^T showed moderate gamma radiation resistance with D_{10} value (dose required to reduce the bacterial population by tenfold) >2 kGy (data not shown). Physiological characteristics of strain SR2-4-2^T within species description and differential characteristics between strain SR2-4-2^T and closely related type strains are summarized in Table 1.

Phylogenetic analysis

The 16S rRNA gene sequence of strain SR2-4-2^T was a continuous stretch of 1440 nucleotides. Based on phylogenetic analysis, the strain SR2-4-2^T belonged to *Flavisolibacter* in the family of *Chitinophagaceae* (class *Sphingobacteriia*). It shared the highest sequence similarity with *F. ginsengisoli* Gsoil 643^T (96.4%), followed by *F. ginsengiterrae* Gsoil 492^T (96.3%) and *F. rigui* 02SUJ3^T (93.0%). The strain SR2-4-2^T

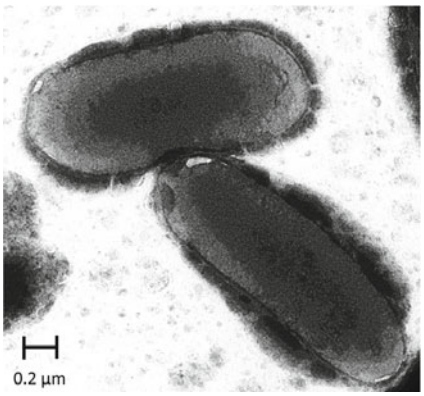

Fig. 1. Transmission electron microcopy of strain SR2-4-2^T. The cell was grown on R2A agar for 3 days at 30°C. Bar, 0.2 μm.

belonged to the genus *Flavisolibacter* linage with high bootstrap values in both NJ and ML phylogenetic trees (Fig. 2 and Supplementary data Figs. S1 and S2).

Chemotaxonomic and genomic analyses

The predominant cellular fatty acids of strain SR2-4-2^T were iso-$C_{15:0}$ (26.4%) and iso-$C_{17:0}$ 3OH (10.7%). It also had moderate to minor amounts of $C_{16:0}$ (8.9%), iso-$C_{15:1}$ G (7.2%), summed feature (composed of $C_{16:1}$ ω6c/$C_{16:1}$ ω7c; 6.8%), $C_{17:1}$ ω6c (6.1%), anteiso-$C_{15:0}$ (5.9%), anteiso-$C_{15:1}$ A (5.3%), summed feature 4 (anteiso-$C_{17:1}$D /iso-$C_{17:1}$I; 5.3%), summed feature 1 (composed of $C_{13:0}$ 3OH/iso-$C_{15:1}$ H; 3.6%), iso-$C_{16:0}$ (3.5%), $C_{16:0}$ 2OH (2.4%), and iso-$C_{14:0}$ (1.9%). The

Table 1. Differential characteristics between strain SR2-4-2^T and other members of *Flavisolibacter*

Strains: 1, SR2-4-2^T; 2, *Flavisolibacter gensengisoli* KCTC 12657^T; 3, *Flavisolibacter ginsengiterrae* KCTC 12656^T; 4, *Flavisolibacter rigui* KCTC 23328^T. All data were obtained from this study. All strains are Gram-staining-negative and non-motile. +, positive; -, negative; w, weak positive.

Characteristic	1	2	3	4
Morphology	rod	rod	rod	curved rod
Colony color	pale yellow	pale yellow	pale yellow	pale yellow
Size (μm):				
Length	1.5–2.5	3.0–6.0	0.6–1.0	1.0–2.0
Width	1.0–1.2	0.3–0.7	0.3–0.7	0.3–0.5
Oxidase activity	+	-	+	-
Catalase activity	-	+	-	+
Production of acid from glucose	w	+	-	-
Growth at :				
15°C	-	-	-	+
25°C	+	+	w	+
35°C	+	+	+	-
37°C	-	+	-	-
pH 6	w	-	-	+
2% NaCl	w	-	+	-
Nitrate reduction	-	+	-	-
Hydrolysis of :				
Casein	+	-	-	+
Gelatin	-	+	+	-
Enzyme activity (API ZYM) :				
N-Acetyl-β-glucosaminidase	+	+	+	-
Arginine dihydrolase	+	+	-	-
α-Chymotrypsin	+	+	+	-
Esterase (C4)	+	w	+	-
Esterase (C8)	-	w	+	-
α-Fucosidase	+	-	-	-
α-Galactosidase	+	w	+	-
β-Galactosidase (ONPG)	+	w	+	-
α-Glucosidase (starch hydrolysis)	+	+	+	-
β-Glucosidase (Esculin hydrolysis)	+	+	+	+
β-Glucosidase	+	-	+	+
Leucine arylamidase	+	+	+	-
α-Mannosidase	+	-	+	-
Naphtol-AS-BI-phosphohydrolase	-	-	+	-
Trypsin	+	-	-	-
Valine arylamidase	+	+	+	-
Assimilation (API 20NE) :				
Adipate	-	w	-	-
Gluconate	-	w	+	-
L-Malate	-	w	-	-
L-Arabinose	-	-	+	-
D-Glucose	+	w	w	-
D-Maltose	-	-	+	-
D-Mannose	+	-	+	-
N-Acetyl-D-glucosamine	+	-	+	-
G+C content	45.0	42.7	43.0	46.4

strain SR2-4-2^T could be differentiated from members of *Flavisolibacter* based on quantitative differences in the fatty acids (Table 2). The strain SR2-4-2^T had MK-7 as the predominant quinone, similar to other members of the genus *Flavisolibacter* (Yoon and Im, 2007; Baik *et al*., 2014). Strain SR2-4-2^T contained major amounts of phosphatidylethanolamine (PE) and minor amounts of unknown amino lipids (Supplementary data Fig. S3). The polar lipid profile of strain SR2-4-2^T was similar to that of other members of the genus *Flavisolibacter* (Baik *et al*., 2014). The G + C content of genomic DNA from strain SR2-4-2^T was 45.0%.

Taxonomic conclusion

Strain SR2-4-2^T showed typical features of genus *Flavisolibacter* with MK-7 as the predominant respiratory quinone, iso-$C_{15:0}$ and iso-$C_{17:0}$ 3OH as the major fatty acids, and PE as the major polar lipid. This strain SR2-4-2^T could be distinguished from other members of *Flavisolibacter* because it had the ability to produce α-fucosidase and trypsin. Based on the phylogenetic, chemotaxonomic, and phenotypic data, we conclude that the strain SR2-4-2^T is a representative novel species for which the name of *Flavisolibacter swuensis* sp. nov. is proposed.

Description of *Flavisolibacter swuensis* sp. nov.

Flavisolibacter swuensis (*swu.en'sis*. N.L. masc. adj. *swuensis* of or belonging to SWU, Seoul Women's University, where bacterial strain was isolated).

It is a Gram-staining-negative, aerobic, non-motile, non-endospore-forming, and rod-shaped (1.5–2.5 × 1.0–1.2 mm) bacterium when grown on R2A agar plates at 30°C for 2 days. Its growth occurs on R2A, but not on TSA, LB, or NA. Its growth can occur at temperature of 25–35°C (optimum, 30°C), pH 7–8 (optimum, pH7). It can tolerate up to 1.5% NaCl (w/v). It is positive for oxidase but negative for catalase. It is positive for hydrolysis of aesculin, starch and casein, but negative for chitin, DNA, gelatin, Tween 80, and xylan. It is negative for nitrate reduction and production of indole. Weak acid is produced from glucose (API 20NE).

In tests with API Zym and 20NE system, positive reactions were observed with N-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, arginine dihydrolase, α-chymotrypsin, cystine arylamidase, esterase (C4), α-fucosidase, β-galactosidase (ONPG), α-galactosidase, α-glucosidase, β-glucosidase, leucine arylamidase, α-mannosidase, trypsin, and valine arylamidase while negative reactions were observed with esterase (C8), lipase (C14), *β*-glucuronidase, naphtol-AS-BI-phosphohydrolase, and urease. In API 20NE

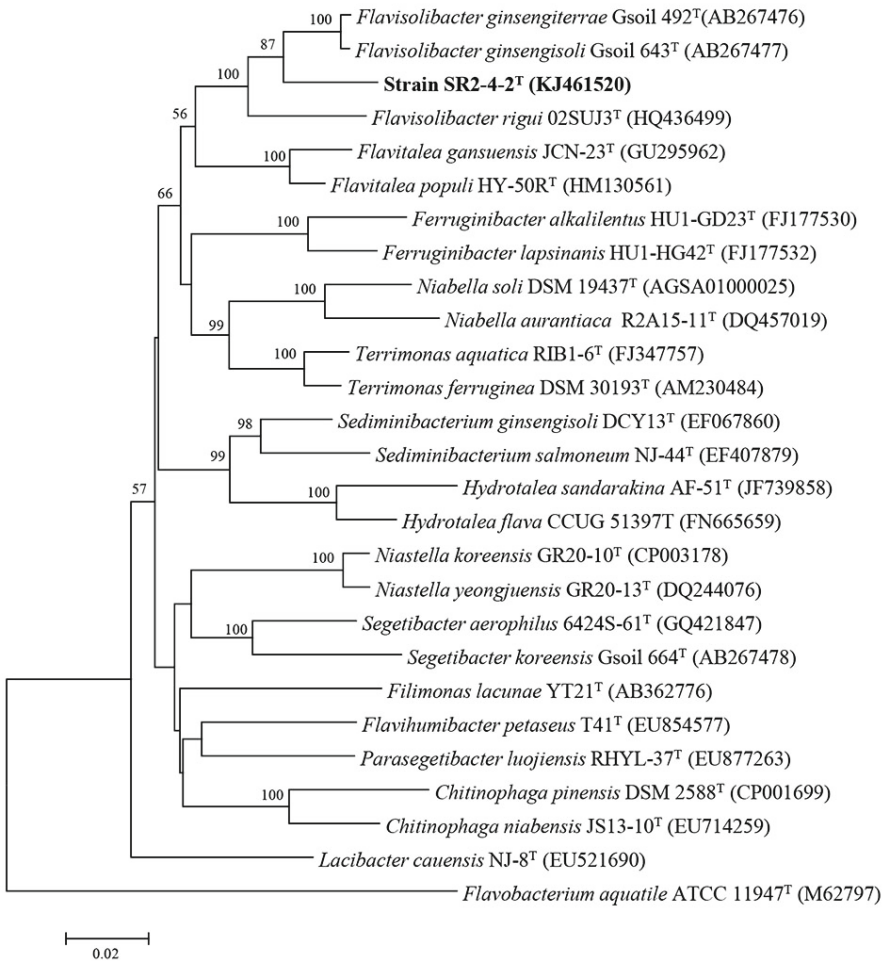

Fig. 2. Neighbor-Joining (NJ) phylogenetic tree based on 16S rRNA gene sequences of strain SR2-4-2^T and representatives of related taxa. Numbers at the nodes indicate the bootstrap values (greater than 50%) expressed as percentage of 1,000 replicates. Bar represents 0.02 substitutions per nucleotide position.

Table 2. Cellular fatty acid profiles of strain SR2-4-2^T and other members of the genus *Flavisolibacter*

Strains: 1, SR2-4-2^T; 2, *Flavisolibacter gensengisoli* KCTC 12657^T; 3, *Flavisolibacter ginsengiterrae* KCTC 12656^T; 4, *Flavisolibacter rigui* KCTC 23328^T.

All strains were grown on R2A agar plates at 30°C for 2 days. Tr: trace (less than 1.0%)

Fatty acids	1	2	3	4
Saturated				
13:0 iso	Tr	Tr	1.0	1.9
14:0	ND	Tr	Tr	2.2
14:0 iso	1.9	1.3	Tr	ND
15:0 iso	**26.4**	**25.7**	**33.4**	**28.1**
15:0 anteiso	5.9	11.0	9.0	7.2
16:0	8.9	7.3	4.8	7.9
16:0 iso	3.5	3.2	1.1	2.9
16:0 2OH	2.4	1.0	ND	ND
17:0 iso	Tr	Tr	2.3	4.1
17:0 2OH	Tr	Tr	Tr	1.6
17:0 iso 3OH	**10.7**	**8.7**	**10.6**	**12.0**
Unsaturated				
14:1 *ω5c*	Tr	Tr	Tr	1.2
15:1 iso G	7.2	4.2	7.2	11.6
15:1 anteiso A	5.3	4.1	3.9	6.1
16:1 *ω5c*	Tr	Tr	Tr	1.6
17:1 *ω6c*	6.1	4.6	1.2	Tr
Summed Feature 1 (13:0 3OH/15:1 i H)	3.6	4.4	4.5	ND
Summed Feature 3 (16:1 *ω6c*/16:1 *ω7c*)	6.8	11.3	9.9	2.1
Summed Feature 4 (17:1 anteiso B/ iso I)	5.3	2.8	4.4	2.1
Summed Feature 9 (17:1 iso *ω9c* /16:0 10-methyl)	Tr	0.9	1.5	3.2

system, N-acetyl-D-glucosamine, D-mannose, and D-glucose were assimilated. However, adipate, L-arabinose, caprate, citrate, gluconate, L-malate, D-maltose, D-mannitol, and phenyl acetate were not assimilated.

The predominant cellular fatty acids of SR2-4-2^T are iso-$C_{15:0}$ and iso-$C_{17:0}$ 3OH. MK-7 is its predominant quinone. The polar lipid profile consists major amounts of phosphatidylethanolamine. Its DNA G+ C content is 45.0%.

The type strain SR2-4-2^T (=JCM 19974^T =KEMB 9004-156^T) was isolated from a gamma ray-irradiated soil collected at Seoul Women's University (GPS; N 37° 37′ 41″ E 127° 5′ 25″), South Korea.

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