Snuella lapsa gen. nov., sp. nov., isolated from tidal flat sediment

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A yellow-coloured, rod-shaped, Gram-reaction-negative, aerobic bacterial strain, designated JC2132^T, was isolated from a tidal flat sediment sample from Ganghwa Island, Korea. The isolate required sea salts for growth. Cells produced non-diffusible carotenoid pigments, but flexirubin-type pigments were absent. Gliding motility was observed. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain JC2132^T represented a distinct phyletic line that reflected a novel generic status within the family *Flavobacteriaceae* with relatively low sequence similarities (<95%) to members of other genera with validly published names. The predominant isoprenoid quinone (MK-6) and DNA G+C content (35 mol%) were consistent with assignment of the isolate to the family *Flavobacteriaceae*, but overall phenotypic traits demonstrated that strain JC2132^T was not closely affiliated with any previously described genera. Based on taxonomic data obtained using a polyphasic approach, it is proposed that strain JC2132^T represents a novel species in a new genus belonging to the family *Flavobacteriaceae*, for which the name *Snuella lapsa* gen. nov., sp. nov. is proposed; the type strain of the type species is JC2132^T (=KACC 14152^T=JCM 17111^T).

The family *Flavobacteriaceae*, proposed by Reichenbach (1991) and emended by Bernardet *et al.* (1996, 2002), is one of the major groups in the phylum ‘*Bacteroidetes*’ and currently accommodates 87 genera with validly published names. It is known that bacteria belonging to this family have diverse ecological niches and dynamic physiological features even within a genus (Bernardet & Nakagawa, 2006). A yellow-pigmented flavobacterial strain, designated JC2132^T, was isolated from tidal flat sediment and was the subject of taxonomic study according to the minimal standards for describing new taxa of the family *Flavobacteriaceae* (Bernardet *et al.*, 2002). On the basis of evidence obtained using a polyphasic approach, the isolate represents a novel species in a new genus in the family *Flavobacteriaceae*.

Strain JC2132^T was isolated from a tidal flat sediment sample of Ganghwa Island, Korea (37° 35′ 32″ N 126° 27′ 25″ E), using a standard dilution plating method on marine agar 2216 (MA; Difco). The isolate was routinely cultured on MA and maintained as glycerol suspensions (20%, w/v) at –80 °C. *Yeosuana aromativorans* GW1-1^T was selected as a reference strain and evaluated under identical experimental conditions to those for strain JC2132^T.

The 16S rRNA gene was enzymically amplified from a single colony. Primers, PCR conditions and sequencing methods have been described previously elsewhere (Chun & Goodfellow, 1995). Preliminary sequence identification against 16S rRNA gene sequences held in the EzTaxon database of prokaryotic type strains with validly published names (Chun *et al.*, 2007) indicated that this isolate belonged to the family *Flavobacteriaceae*. The nearly complete 16S rRNA gene sequence of strain JC2132^T (1421 bp) was aligned manually against sequences of representatives of the family *Flavobacteriaceae*. The regions available for all sequences (positions 93–182, 213–820 and 850–1437; *Escherichia coli* numbering system) showed unambiguous alignment and were used to construct phylogenetic trees. Alignment and neighbour-joining tree (Saitou & Nei, 1987) analyses were carried out using the program jPHYDIT (Jeon *et al.*, 2005). An evolutionary distance matrix for the neighbour-joining tree was generated according to the model of Jukes & Cantor (1969) and the resultant tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. A Bayesian inference was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) with the General Time Reversible evolutionary model with a gamma-distributed rate variation. The program was run for 20 000 000 generations with a sample frequency of 100 and a burn-in of 20 000. A maximum-likelihood tree

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JC2132^T is HM475133.

(Felsenstein, 1993) was constructed using PAUP 4.0 (Swofford, 1998) with a heuristic search option and 1000 replicates. On the basis of 16S rRNA gene analyses, strain JC2132^T showed highest sequence similarity to *Gaetbulibacter marinus* IMCC1914^T (94.9 %), followed by *Yeosuana aromativorans* GW1-1^T (94.6 %), *Meridianimaribacter flavus* NH57N^T (94.1 %), *Flaviramulus basaltis* H35^T (94.0 %), *Gaetbulibacter saemankumensis* SMK-12^T (93.9 %), *Olleya marilimosa* CAM030^T (93.5 %), members of the genus *Mariniflexile* (93.3–93.4 %), members of the genus *Tamlana* (93.2–93.4 %) and *Algibacter lectus* KMM 3902^T (93.1 %). No members of any other taxon showed more than 93 % sequence similarity to the isolate. Phylogenetic analysis revealed that strain JC2132^T could be clearly distinguished from members of any genera with validly published names (Fig. 1). Strain JC2132^T formed a distinct phyletic line within the radiation of the family *Flavobacteriaceae* and the branching position varied depending on the tree-making algorithm used. The genera *Yeosuana* and *Meridianimaribacter* were recovered as sister groups of the isolate in the neighbour-joining tree and in the maximum-likelihood and Bayesian trees, respectively, but the rigidity of the groupings was not supported by bootstrap value or posterior probability. A further monophyletic clade composed of strain JC2132^T and the genera *Yeosuana*, *Meridianimaribacter* and *Gaetbulibacter* was shown in the maximum-likelihood and Bayesian trees, but not in the neighbour-joining tree. Thus, based on 16S rRNA gene sequence analyses, strain JC2132^T appears to represent a novel species in a new genus of the family *Flavobacteriaceae*.

Growth was tested on seawater-supplemented bacterial media including cetrimide agar (Difco), MacConkey agar (Difco) and yeast extract-free ZoBell agar (Zobell, 1941). Growth at various temperatures (5–50 °C at intervals of 5 °C), pH (pH 5–10.5 at intervals of 0.5 pH units; adjusted with 10 M KOH or HCl) and NaCl or sea salts concentrations [0, 0.5, 1, 2, 3, 4, 5, 7, 10, 12, 15 and 20 % (w/v)] was determined using synthetic ZoBell medium. Growth under anaerobic and microaerophilic conditions was assessed using anaerobically prepared MA in an anaerobic chamber (10 % CO₂, 10 % H₂, 80 % N₂; Sheldon Manufacturing) and CampyPak Plus systems (5–15 % O₂ and 5–12 % CO₂; BBL).

Cellular morphology and motility were examined after growth on MA at 30 °C for 2 days by transmission electron and phase-contrast microscopy, respectively. Gliding motility was observed by direct phase-contrast microscopic examination of cells initially grown in marine broth 2216 (Difco) at 30 °C for 2 days and subsequently incubated for 16, 48 and 72 h on microscope slides coated with MA (0.7 % agar), according to the method described by Bowman (2000). The presence of flexirubin-type pigments was determined by flooding the cell mass taken from agar plates with 20 % (w/v) KOH and confirmed by examining bathychromatic shift of the absorbance spectrum of ethanol and alkaline-ethanol extracts of lysed cells (Weeks, 1981).

Standard physiological and biochemical tests were performed as described previously (Smibert & Krieg, 1994). Hydrolysis of alginic acids (0.5 %; Sigma), casein (5 % skimmed milk; Difco), microcrystalline cellulose (0.5 %; Sigma), chitin (0.5 %; Sigma), egg yolk (5 %; Oxoid), elastin (0.5 %; Sigma), pectin (0.5 %; Sigma), starch (0.2 %; Difco), Tween 20 (1 %; Sigma), Tween 40 (1 %; Junsei), Tween 60 (1 %; Junsei) and Tween 80 (1 %; Sigma) was tested using MA as basal medium. Decomposition of adenine (0.5 %; Sigma), hypoxanthine (0.5 %; Sigma), L-tyrosine (0.5 %; Sigma) and xanthine (0.4 %; Sigma) was tested using MA according to Gordon *et al.* (1974). Other enzymic activities were determined using API 20NE, API 20E and API ZYM kits (bioMérieux). Acid production from carbohydrates was tested by using API 50CH with CHB/E medium (bioMérieux) supplemented with half-strength seawater. API kits were inoculated with a heavy bacterial suspension in half-strength artificial seawater and data were recorded for up to five consecutive days.

DNA G+C content was determined by the thermal denaturation method (Marmur & Doty, 1962). Menaquinones were isolated from 3-day-old cells according to Minnikin *et al.* (1984) and analysed by HPLC (Waters) as described by Collins (1985). For fatty acid analysis, strain JC2132^T and the reference strain were grown on MA at 30 and 25 °C, respectively, for 2 days. Extraction of fatty acid methyl esters and their separation by GC were performed by using the Instant FAME method of the Microbial Identification System (MIDI) version 6.1 and the RTSBA6 6.10 database. The fatty acids of strain JC2132^T were iso-C_{15:0} (18.6 %), iso-C_{15:1} G (17.2 %), iso-C_{17:0} 3-OH (16.4 %), iso-C_{15:0} 3-OH (8.4 %), summed feature 3 (comprising C_{16:1}ω6c and/or C_{16:1}ω7c; 8.4 %), iso-C_{16:0} 3-OH (5.5 %), anteiso-C_{15:0} (4.9 %), summed feature 9 (C_{16:0} 10-methyl and/or iso-C_{17:1}ω9c; 3.3 %), C_{15:1}ω6c (2.7 %), anteiso-C_{15:1} (1.7 %), C_{16:0} (1.6 %), C_{17:1}ω6c (1.5 ± 0.3 %), C_{15:0} 2-OH (1.3 ± 0.0 %), iso-C_{13:0} (1.2 ± 0.0 %), iso-C_{14:0} (1.2 ± 0.1 %) and C_{16:0} 3-OH (1.1 ± 0.0 %).

Results of morphological, cultural, biochemical, physiological and chemotaxonomic tests are presented in the species description.

Phylogenetic analyses based on 16S rRNA gene sequences showed that strain JC2132^T represents a distinct phyletic line that reflects novel genus status. Overall phenotypic traits also demonstrated that strain JC2132^T was not closely affiliated with members of any recognized genera (Table 1). Thus, based on data obtained in this polyphasic study, strain JC2132^T represents a novel species in a new genus belonging to the family *Flavobacteriaceae*, for which the name *Snuella lapsa* gen. nov., sp. nov. is proposed.

Description of *Snuella* gen. nov.

Snuella (Snu.el'la. N.L. fem. dim. n. *Snuella* arbitrary name derived from the acronym of the Seoul National University, SNU, where this taxon was studied).

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences. Numbers at the nodes are percentages of bootstrap support (>50%) from 1000 resampled datasets. Letters at the nodes indicate that the corresponding nodes (groupings) were recovered also in Bayesian (B) and/or in maximum-likelihood (L) tree-inferring methods. *Helicobacter pylori* NCTC 11637^T (Z25741) was used as an outgroup. Bar, 0.05 nt substitutions per position.

Gram-reaction-negative, oxidase- and catalase-positive and aerobic. Cells are rod-shaped with rounded ends and gliding ability. Spores are not formed. Produces non-diffusible carotenoid pigments, but flexirubin-type pigments are absent. Requires sea salts, but not yeast extract

for growth. Major isoprenoid quinone is MK-6. Predominant cellular fatty acids are iso-C_{15:0}, iso-C_{15:1} G and iso-C_{17:0} 3-OH. Maximum absorption peak of pigments is at 452 nm and the next shoulder peak is at 480 nm. A member of the family *Flavobacteriaceae*, class

Table 1. Characteristics that differentiate strain JC2132^T from other phylogenetically related genera in the family *Flavobacteriaceae*

Taxa: 1, strain JC2132^T (data from this study); 2, *Yeosuana aromatorans* GW1-1^T (all data from this study except the DNA G+C content and isoprenoid quinone); 3, *Gaetbulibacter* ($n=2$; Jung *et al.*, 2005; Yang & Cho, 2008); 4, *Gelidibacter* ($n=4$; Bowman *et al.*, 1997; Bowman & Nichols, 2005; Macián *et al.*, 2002); 5, *Meridianimaribacter flavus* NH57N^T (Wang *et al.*, 2010); 6, *Subsaxibacter broadyi* P7^T (Bowman & Nichols, 2005); 7, *Subsaximicrobium* ($n=2$; Bowman & Nichols, 2005). n , Number of type strains in each genus; +, positive; –, negative; w, weakly positive; ND, no data available; v, varies among different species within the same genus. All taxa are positive for catalase and leucine arylamidase and negative for β -glucuronidase activity and production of flexirubin-type pigments (no data available for *Meridianimaribacter flavus*), indole or H₂S.

Characteristic	1	2	3	4	5	6	7
DNA G+C content (mol%)	35	51.4*	34.7–38.1	36–42	32.7	35	38–41
Major isoprenoid quinone(s)	MK-6	MK-5, MK-6*	MK-6	MK-6	MK-6	MK-6	MK-6
Major fatty acids	i-C _{15:0} , i-C _{15:1} , i-C _{17:0} 3-OH	i-C _{15:0} , i-C _{16:0} 3-OH, i-C _{17:0} 3-OH	i-C _{15:0} , i-C _{15:1} , i-C _{17:0} 3-OH	ai-C _{15:0} , ai-C _{15:1}	i-C _{15:0} , i-C _{15:1} , i-C _{17:0} 3-OH	ai-C _{15:0} , ai-C _{15:1} , C _{18:1} ω 9c	ai-C _{15:0} , ai-C _{15:1}
Growth requirements:							
Sea salts†	+	–	–	–	–	–	–
Oxygen	+	+	v	+	+	+	+
Yeast extract	–	–	ND	–	ND	+	–
Gliding motility	+	+‡	v	+	+	+	+
Oxidase activity	+	+	v	–	+	ND	ND
Urease activity	–	–	–	v	–	–	–
Acid production from glucose	–	–	v	+	–	–	–
Acid production from carbohydrates	+	+	+	+	ND	–	–
Production of acetoin	–	+	ND	ND	+	ND	ND
Nitrate reduction	+	–	v	–	–	–	–
Degradation of:							
Casein	–	–	v	v	–	–	+
Egg yolk	–	Clear§	ND	v	ND	–	–
Aesculin	+	+	+	+	+	w	+
Gelatin	–	+	–	v	+	+	+
Starch	+	+	v	v	w	–	+
Tween 20	+	–	+	ND	–	ND	ND
Tween 40	+	–	–	ND	w	ND	ND
Tween 60	+	–	–	ND	ND	ND	ND
Tween 80	+	+	–	v	–	+	+
L-Tyrosine	+	+	+	v	ND	+	v
API ZYM/API ID32A:							
Alkaline phosphatase	+	+	+	v	+	+	+
Esterase (C4), esterase lipase (C8)	–	–	+	ND	+	ND	ND
Lipase (C14)	–	–	v	ND	–	ND	ND
Valine arylamidase, acid phosphatase	+	+	+	ND	+	ND	ND
Cystine arylamidase	–	+	+	ND	w	ND	ND
Trypsin	w	+	v	ND	w	ND	ND
α -Chymotrypsin	–	–	v	ND	+	ND	ND
Naphthol-AS-BI-phosphohydrolase	+	+	v	ND	+	ND	ND
α -Galactosidase	–	–	–	v	–	–	–
β -Galactosidase	+	+	–	v	+	–	–
α -Glucosidase	w	–	v	v	w	–	+
β -Glucosidase	–	–	v	v	+	–	–
N-Acetyl- β -glucosaminidase	+	+	v	+	–	–	–

Table 1. cont.

Characteristic	1	2	3	4	5	6	7
α -Mannosidase, α -fucosidase	—	—	—	ND	—	ND	ND

*Data from Kwon *et al.* (2006).

†Cannot grow in the presence of NaCl alone.

‡Data differs from that in a previous report (Kwon *et al.*, 2006).

§Egg yolk decomposition was negative but a clear zone was formed around colonies.

||Positive in the API ZYM and API 20E tests, but negative in the API 20E test.

Flavobacteria, phylum ‘*Bacteroidetes*’. The type species is *Snuella lapsa*.

Description of *Snuella lapsa* sp. nov.

Snuella lapsa [lap’sa. L. fem. part. adj. *lapsa* (from L. v. *labor* to glide) gliding].

In addition to properties given in the genus description, grows under aerobic and microaerobic conditions, but not under anaerobic conditions. Cells are rod-shaped with rounded ends and approximately $0.6\text{--}0.7 \times 1.5\text{--}2.7\ \mu\text{m}$. Colonies are yellow-coloured, convex, translucent, glistening and circular with entire margins. Gliding is observed on agar plates. Does not grow on cetrimide or MacConkey agar. Growth occurs at pH 6–9 (optimum, pH 7) and 10–40 °C (optimum, 30 °C). Requires a sea salts concentration of 1–7 % (optimum, 3–4 %) for growth and cannot grow on sea-salts-free ZoBell medium supplemented with NaCl only. Reduces nitrate to nitrogen. Does not produce acetoin, indole or H₂S. Does not utilize citrate. Acid is not produced from glucose. Decomposes aesculin, starch, Tweens 20, 40, 60 and 80, and L-tyrosine, but not agar, adenine, alginic acid, casein, cellulose, chitin, egg yolk, elastin, gelatin, pectin, hypoxanthine or xanthine. Positive for alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -galactosidase and *N*-acetyl- β -glucosaminidase, and weakly positive for trypsin and α -glucosidase. Negative for urease, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, α -chymotrypsin, α -galactosidase, β -glucuronidase, β -glucosidase, α -mannosidase and α -fucosidase. β -Galactosidase activity is positive in API ZYM and API 20NE kits, but negative in the API 20E kit. In the API 50CH system, produces acid from D-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, aesculin ferric citrate, salicin, cellobiose, maltose, lactose (bovine origin), melibiose, sucrose, trehalose, raffinose, starch and gentiobiose; acid is produced weakly from methyl β -D-xylopyranoside, glycogen and turanose; and no acid is produced from glycerol, erythritol, L-arabinose, D-ribose,

L-xylose, D-adonitol, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, inulin, melezitose, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate. The fatty acids are composed of iso-C_{15:0}, iso-C_{15:1} G, iso-C_{17:0} 3-OH, iso-C_{15:0} 3-OH, summed feature 3 (comprising C_{16:1}ω6c and/or C_{16:1}ω7c), iso-C_{16:0} 3-OH, anteiso-C_{15:0}, summed feature 9 (comprising C_{16:0} 10-methyl and/or iso-C_{17:1}ω9c), C_{15:1}ω6c, anteiso-C_{15:1}, C_{16:0}, C_{17:1}ω6c, C_{15:0} 2-OH, iso-C_{13:0}, iso-C_{14:0} and C_{16:0} 3-OH.

The type strain is JC2132^T (=KACC 14152^T=JCM 17111^T), isolated from a tidal flat sediment sample from Ganghwa Island, Korea. The DNA G+C content of the type strain is 35 mol%.

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