Soonwooa buanensis gen. nov., sp. nov., a member of the family *Flavobacteriaceae* isolated from seawater

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A Gram-negative, non-motile, non-gliding, strictly aerobic, pale yellow colony-forming, rod-shaped bacterial strain, designated HM0024^T, was isolated from coastal seawater of the Yellow Sea and subjected to a polyphasic taxonomy study. Based on 16S rRNA gene sequence similarities, strain HM0024^T was most closely related to *Chryseobacterium balustinum* LMG 8329^T (94.4 %), *Chryseobacterium scophthalmum* LMG 13028^T (94.4 %), *Chryseobacterium piscium* LMG 23089^T (94.3 %) and *Elizabethkingia meningoseptica* ATCC 13253^T (94.0 %) and shared less than 92 % sequence similarity with other members of the family *Flavobacteriaceae*. Phylogenetic analyses showed that strain HM0024^T formed an independent phyletic line of descent within the family *Flavobacteriaceae*. The DNA G+C content of the strain was 29.6 mol% and its major cellular fatty acids (>10 %) were iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{17:1}\omega7c$ and summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$). The major isoprenoid quinone was MK-6 and the major polar lipids were phosphatidylethanolamine and several aminolipids. Strain HM0024^T was differentiated from phylogenetically related members of the family by having lower DNA G+C content, larger proportions of summed feature 3, anteiso-$C_{15:0}$ and iso-$C_{16:0}$ 3-OH and particular phenotypic characteristics. On the basis of phenotypic and phylogenetic data, strain HM0024^T is classified as a representative of a novel genus and species, for which the name *Soonwooa buanensis* gen. nov., sp. nov. is proposed. The type strain of *Soonwooa buanensis* is HM0024^T (=KCTC 22689^T =CECT 7503^T).

The family *Flavobacteriaceae* (Bernardet *et al.*, 2002; Bernardet & Nakagawa, 2006), a lineage in the phylum *Bacteroidetes*, currently comprises more than 85 genera (http://www.bacterio.cict.fr). Most members of the family have been isolated from diverse marine environments including the deep sea (Einen & Øvreås, 2006; Romanenko *et al.*, 2007), sediment (Khan *et al.*, 2008), Antarctic water (Bowman *et al.*, 1997; Macián *et al.*, 2002) and marine plants or animals (Nedashkovskaya *et al.*, 2005; Bae *et al.*, 2007).

A seawater sample from the coastal surface of the Yellow Sea (Buan beach, Korea; 35° 40′ N 126° 31′ E) was serially diluted, spread onto nutrient agar (NA; BD Difco) and incubated aerobically at 30 °C. After 2 days of incubation, a yellow colony-forming strain, designated HM0024^T, was isolated and stored as a suspension in nutrient broth (NB; BD Difco) with 15 % (v/v) glycerol. For further taxonomic studies, the strain was maintained routinely at 30 °C on NA.

The methods employed for DNA extraction, PCR amplification and sequencing of the 16S rRNA gene were essentially the same as described in a previous study (Cho & Giovannoni, 2003). The almost-complete 16S rRNA gene sequence (1449 bp) of strain HM0024^T was aligned automatically using the PT server implemented in the ARB software package (Ludwig *et al.*, 2004) and the alignment was checked manually. 16S rRNA gene sequence similarities were calculated from the sequence alignment in the ARB package and were confirmed by the EzTaxon server (Chun *et al.*, 2007). Sequence comparisons showed that strain HM0024^T was closely associated with several members of the genera *Chryseobacterium* and *Elizabethkingia* of the family *Flavobacteriaceae*. The strain was most closely related to *Chryseobacterium balustinum* LMG 8329^T (94.4 %), *Chryseobacterium scophthalmum* LMG 13028^T (94.4 %), *Chryseobacterium piscium* LMG

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23089^T (94.3 %) and *Elizabethkingia meningoseptica* ATCC 13253^T (94.0 %) and shared 90.2–94.3 % sequence similarity with other *Chryseobacterium* strains, 92.8 % with *Elizabethkingia miricola* GTC 862^T, 92.2 % with *Bergeyella zoohelcum* ATCC 43767^T, 91.7 % with *Epilithonimonas tenax* EP105^T and 91.6–92.0 % with *Riemerella* strains. To clarify the phylogenetic position of the novel strain, 1178 nucleotide positions that could be unambiguously aligned were used to generate phylogenetic trees using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) algorithms in PAUP* version 4.0b10 (Swofford, 2002). The robustness of the neighbour-joining and maximum-parsimony trees was evaluated by bootstrap analyses with 1000 resamplings. The three phylogenetic trees, summarized in Fig. 1, showed that strain HM0024^T formed a distinct evolutionary line of descent in the *Chryseobacterium–Bergeyella–Riemerella* group in the family *Flavobacteriaceae* (Vandamme *et al.*, 1994; Bernardet *et al.*, 1996).

The two closest neighbours of strain HM0024^T in each of the genera *Chryseobacterium* and *Elizabethkingia*, *C. balustinum* KCTC 2903^T, *C. scophthalmum* KCTC 2907^T, *E. meningoseptica* KCTC 2906^T and *E. miricola* KCTC 12492^T, were grown under the same conditions as strain HM0024^T and used as reference strains in the phenotypic study. Gram reaction, cell morphology and size, colony description, presence of flagellar and gliding motility, catalase and oxidase activities, presence of flexirubin-type pigments and absorption spectra of cellular pigments were determined by methods described by Yang & Cho (2008) and Bernardet *et al.* (2002) except that bacterial cultures were grown on NA or in NB at 30 °C for 2 days. The temperature range and optimum for growth were measured on NA at 4, 10–30 (at intervals of 5 °C), 37 and 42 °C. The pH range and optimum for growth were determined at pH 4.0–10.0 (at intervals of 1 pH unit) in NB in which the pH had been adjusted using 0.1 M HCl or 0.1 M Na_2CO_3. Growth in the presence of 0.5 and 1.0–10.0 % NaCl (at 1.0 % intervals) was assessed in NB. Growth under anaerobic conditions was tested on NA using a gas pack and anaerobic jar (BD Difco) at 30 °C for 5 days. Hydrolysis of casein (50 %, v/v, skimmed milk; Difco), CM-cellulose (0.5 %, v/v; Sigma), crystalline cellulose (1 % Whatman No. 1 filter paper) and starch (0.2 %, w/v) was tested using NA as the basal medium. Growth was tested on MacConkey agar (BD Difco) and DNA hydrolysis was assessed on DNase test agar (BD Difco). Production of H_2S was investigated using triple-sugar iron agar (BD Difco). Susceptibility to antimicrobial agents was tested on tryptic soy agar (TSA; BD Difco) using discs containing (μg): ampicillin (10), chloramphenicol (25), gentamicin (10), kanamycin (30), penicillin G (10), tetracycline (30) and vancomycin (30). Other biochemical tests and carbon source oxidation tests were performed using API 20E, API 20NE and API ZYM strips (bioMérieux) and GN2 MicroPlates (Biolog), according to the manufacturers' instructions except that inoculants were prepared from bacterial cultures grown on NA at 30 °C for 2 days. The DNA G+C content was determined by using HPLC (Mesbah *et al.*, 1989). For fatty acid methyl ester analysis, biomass was produced on TSA at 30 °C for 2 days. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System. Isoprenoid quinones were isolated using the method of Minnikin *et al.* (1984) and measured by HPLC. Polar lipids were extracted and

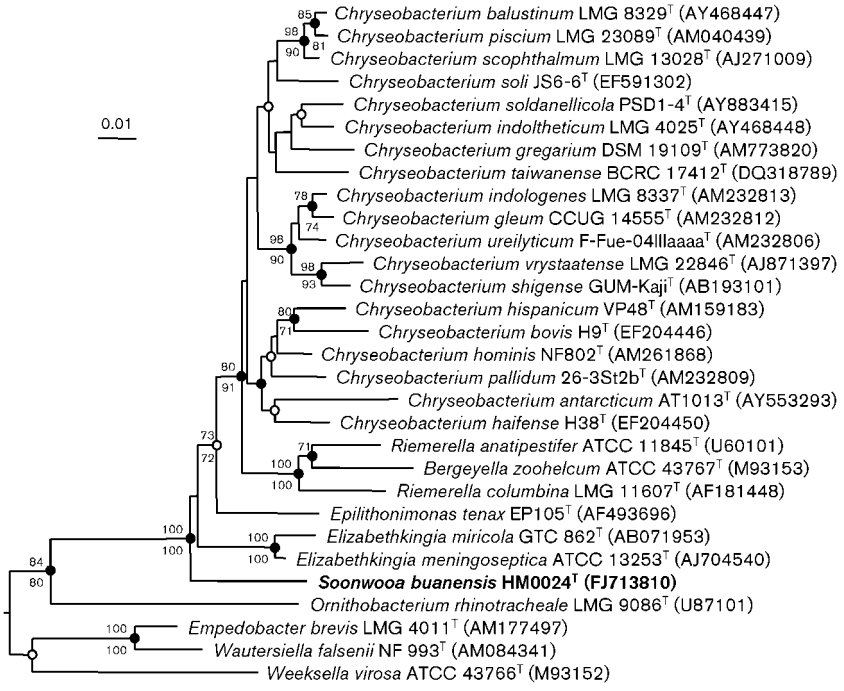

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain HM0024^T in the family *Flavobacteriaceae*. Bootstrap values (>70 %) based on 1000 resamplings are shown for the neighbour-joining (above nodes) and the maximum-parsimony (below nodes) algorithms. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-parsimony and maximum-likelihood algorithms. Open circles indicate that the corresponding nodes were also recovered with either the maximum-parsimony or the maximum-likelihood algorithm. The sequence of *Gilvibacter sediminis* Mok-1-36^T (GenBank accession no. AB255368) was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

examined using two-dimensional TLC (Minnikin *et al.*, 1984).

Cells of strain HM0024^T were Gram-negative, strictly aerobic, chemoheterotrophic, non-motile rods. The absorption spectrum of cellular pigments of strain HM0024^T exhibited absorption maxima at 421, 450 and 474 nm, which indicates the presence of carotenoid pigments (Peterson *et al.*, 1954). Flexirubin-type pigments were determined not to be produced by the results of two different KOH tests: no colour change occurred when colonies were flooded with a 20 % KOH solution and no bathochromic shift occurred when KOH was added to a methanol extract of cellular pigments. These pigment characteristics clearly differentiated strain HM0024^T from the four reference type strains. Other phenotypic characteristics, including carbon source utilization pattern, growth properties and enzyme activities, also differentiated strain HM0024^T from the reference strains (Table 1). The only respiratory quinone detected was MK-6, which is in agreement with those in all other members of the family *Flavobacteriaceae*. The polar lipids of strain HM0024^T were composed of phosphatidylethanolamine, several aminolipids and unknown lipids. The G+C content of the genomic DNA of strain HM0024^T was 29.6 mol%, which was 4–8 mol% lower than that of related strains. The major cellular fatty acids of strain HM0024^T were iso-$C_{15:0}$ (30.6 %), iso-$C_{17:0}$ 3-OH (16.0 %), iso-$C_{17:1}\omega7c$ (13.7 %), summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$; 11.9 %), anteiso-$C_{15:0}$ (6.9 %) and iso-$C_{16:0}$ 3-OH (5.9 %). This pattern differed from those of related strains particularly by having larger proportions of summed feature 3, anteiso-$C_{15:0}$ and iso-$C_{16:0}$ 3-OH (Table 2). Phenotypic characteristics of strain HM0024^T are listed in the genus and species descriptions.

As shown by the low 16S rRNA gene sequence similarity (<94.5 %) to members of related genera, the formation of

Table 1. Differential characteristics of strain HM0024^T and phylogenetically related members of the family *Flavobacteriaceae*

Strains: 1, *Soonwooa buanensis* gen. nov., sp. nov. HM0024^T; 2, *C. balustinum* KCTC 2903^T; 3, *C. scophthalmum* KCTC 2907^T; 4, *E. meningoseptica* KCTC 2906^T; 5, *E. miricola* KCTC 12492^T. Data in parentheses were taken from Kim *et al.* (2005); other data were obtained in this study. +, Positive; V, variable; −, negative; NA, not applicable; ND, no data available.

Characteristic	1	2	3	4	5
Growth at/on:					
15 °C	−	+	+	+	+
MacConkey agar	−	− (+)	−	− (V)	+
Cellular pigmentation					
Carotenoid pigments	+	−	−	−	−
Flexirubin-type pigments	−	+	+	−	−
Absorption peak(s) (nm)	421, 450, 474	450	452	ND	ND
Bathochromic shift (nm)	−	450→476	452→478	NA	NA
Nitrate reduction	−	+	−	−	−
Indole production	−	+	+ (−)	+	− (+)
Acid production from glucose	−	+	−	+	+
Enzyme activities					
β-Galactosidase	−	−	−	+	+
Arginine dihydrolase	+	+	−	+	+
Lysine decarboxylase	+	+	−	+	−
Ornithine decarboxylase	+	+	−	−	+
Urease	−	− (+)	+	−	+
Trypsin	−	+	+	+	+
α-Chymotrypsin	+	−	−	−	−
Utilization of:					
Arabinose	+	−	−	+ (−)	−
D-Arabitol	+	−	−	−	−
i-Erythritol	+	−	−	−	−
L-Fucose	+	−	−	−	−
Lactose	+	+	−	−	−
D-Sorbitol	+	−	−	−	−
Hydrolysis of:					
Starch	+	+ (−)	−	+	+
DNA	+	+	+	+	−
Aesculin	−	+	+	+	+
Gelatin	+	−	−	+	+
DNA G+C content (mol%)	29.6	(34.7)	(34.1)	(37.2)	(35.3)

Table 2. Cellular fatty acid composition of strain HM0024^T and phylogenetically related members of the family *Flavobacteriaceae*

Strains: 1, *S. buanensis* gen. nov., sp. nov. HM0024^T; 2, *C. balustinum* KCTC 2903^T; 3, *C. scophthalmum* KCTC 2907^T; 4, *E. meningoseptica* KCTC 2906^T; 5, *E. miricola* KCTC 12492^T. Values are percentages of total fatty acids and were generated in this study. Strains were grown for 2 days at 30 °C on TSA. Only fatty acids representing >1.0 % of the total fatty acids are shown. tr, Traces (<1.0 %); −, not detected.

Fatty acid	1	2	3	4	5
iso-$C_{13:0}$	tr	1.3	1.1	1.5	2.7
iso-$C_{14:0}$	1.9	−	−	4.1	7.9
anteiso-$C_{15:0}$	6.9	2.0	1.5	1.6	1.2
iso-$C_{15:0}$	30.6	50.9	36.5	37.9	29.5
iso-$C_{15:0}$ 3-OH	3.0	−	3.5	3.8	4.4
iso-$C_{16:0}$	3.6	−	tr	3.1	3.1
iso-$C_{16:0}$ 3-OH	5.9	tr	tr	1.7	2.6
$C_{16:0}$	tr	1.5	1.0	1.1	1.4
iso-$C_{17:0}$	tr	1.5	tr	1.3	tr
iso-$C_{17:0}$ 3-OH	16.0	4.2	16.8	16.3	13.7
iso-$C_{17:1}\omega7c$	13.7	24.6	17.8	2.6	3.1
$C_{18:0}$	1.7	−	tr	−	tr
Summed feature 3*	11.9	−	1.7	2.7	2.1

*Summed features represent groups of two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$.

an independent lineage in phylogenetic trees and several differential biochemical and chemotaxonomic characteristics, strain HM0024^T could not be assigned to any of the known genera in the family *Flavobacteriaceae*. Therefore, strain HM0024^T should be classified as a member of a novel genus and species, for which the name *Soonwooa buanensis* gen. nov., sp. nov. is proposed.

Description of *Soonwooa* gen. nov.

Soonwooa (Soon.woo′a. N.L. fem. n. *Soonwooa* named in memory of Professor Soon-Woo Hong, a microbiologist who founded the first university microbiology department in Korea).

Cells are Gram-negative, strictly aerobic, chemoheterotrophic rods devoid of flagellar and gliding motility. Oxidase- and catalase-positive. Carotenoid pigments are present, but flexirubin-type pigments are absent. The predominant fatty acids are iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{17:1}\omega7c$ and summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$). The major respiratory quinone is MK-6. The major polar lipids are phosphatidylethanolamine and several aminolipids. The DNA G+C content of the type strain of the type species is 29.6 mol%. The genus belongs to the family *Flavobacteriaceae*. The type species is *Soonwooa buanensis*.

Description of *Soonwooa buanensis* sp. nov.

Soonwooa buanensis (bu.an.en′sis. N.L. fem. adj. *buanensis* pertaining to Buan beach, where the type strain was isolated). Displays the following characteristics in addition to those given in the genus description. Cells are about 1.8 μm long and 0.6 μm in diameter. After 2 days of incubation on NA, colonies are circular, slightly convex, glistening and smooth with regular edges and pale yellowish in colour. Growth occurs at 15–30 °C (optimum 30 °C), at pH 6.0–8.0 (optimum pH 7.0) and with 0–1.5 % NaCl (optimum 0.75 %). Carotenoid pigments with absorption spectrum peaks at 421, 450 and 474 nm are found. Does not require NaCl or seawater for growth. Grows on TSA. Does not grow on MacConkey agar. Nitrate is not reduced. Does not produce indole or H_2S. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and DNase activities are present. Hydrolyses starch, gelatin and CM-cellulose, but not casein, aesculin or crystalline cellulose (filter paper). With API ZYM, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, *N*-acetyl-β-glucosaminidase and α-fucosidase activities are present, but lipase (C14), trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase and α-mannosidase activities are absent. Susceptible to ampicillin, penicillin G, chloramphenicol, gentamicin, kanamycin and tetracycline. With GN2 MicroPlates, does not oxidize D-fructose, pyruvic acid methyl ester, γ-hydroxybutyric acid, D-serine, phenylethylamine, putrescine or 2-aminoethanol; all other substrates are oxidized. The major cellular fatty acids (>5 %) are iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{17:1}\omega7c$, summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$), anteiso-$C_{15:0}$ and iso-$C_{16:0}$ 3-OH.

The type strain HM0024^T (=KCTC 22689^T =CECT 7503^T) was isolated from coastal seawater near Buan beach of the Yellow Sea, Korea.

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