Chryseobacterium soli sp. nov. and *Chryseobacterium jejuense* sp. nov., isolated from soil samples from Jeju, Korea

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Two yellow-pigmented bacterial strains, JS6-6^T and JS17-8^T, isolated from soil samples from Jeju, Republic of Korea, were studied to determine their taxonomic positions. The cells of the two bacteria were aerobic, Gram-negative, non-motile, straight rods. 16S rRNA gene sequence analysis indicated that both isolates should be placed in the genus *Chryseobacterium* of the family *Flavobacteriaceae*. Their 16S rRNA gene sequences showed similarities of 93.7–97.5 % to those of type strains of the genus *Chryseobacterium*. The values for DNA–DNA relatedness between both strains and type strains of closely related *Chryseobacterium* species were below 34 %. The fatty acids of the novel strains were similar to those of species of the genus *Chryseobacterium*. Both strains had MK-6 as the predominant respiratory quinone. The DNA G + C contents of strains JS6-6^T and JS17-8^T were 39.9 and 41.4 mol%, respectively. Phylogenetic evidence, together with the DNA–DNA relatedness values and phenotypic characteristics, indicated that strains JS6-6^T and JS17-8^T represent two novel species of the genus *Chryseobacterium*, for which the names *Chryseobacterium soli* sp. nov. and *Chryseobacterium jejuense* sp. nov., respectively, are proposed. The type strains of *Chryseobacterium soli* sp. nov. and *Chryseobacterium jejuense* sp. nov. are JS6-6^T (=KACC 12502^T=DSM 19298^T) and JS17-8^T (=KACC 12501^T=DSM 19299^T), respectively.

The genus *Chryseobacterium* was first created for five species formerly classified as members of the genus *Flavobacterium*, primarily on the basis of 16S rRNA gene sequence analysis (Vandamme *et al.*, 1994). At the time of writing, the genus includes 16 species with MK-6 as the major respiratory quinone and iso-C_{15:0}, iso-C_{17:1}ω9c and iso-C_{17:0} 3-OH as the major fatty acids. Strains belonging to this genus have been found in a wide variety of environments such as soil, plant roots, sludge, fish, raw chicken, sewage, fresh water, marine sediment, freshwater sediment, fermented beverages, dairy products and clinical samples.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains JS6-6^T and JS17-8^T are EF591302 and EF591303, respectively.

The cellular fatty acid profiles of strains JS6-6^T, JS17-8^T and related species of the genus *Chryseobacterium* are presented in a supplementary table available with the online version of this paper.

In the course of studies on the bacterial population in soil samples from Jeju, Republic of Korea, we isolated two yellow-pigmented strains. The soil samples were serially diluted with 0.85 % NaCl (w/v) and the dilutions plated onto R2A agar (Difco). The strains were isolated after incubation for 4 days at 28 °C. Subsequently, the isolates were maintained both on R2A medium at 4 °C and in R2A broth supplemented with 30 % (v/v) glycerol at –80 °C.

Morphological, physiological and biochemical analyses were performed at 28 °C with R2A as the basal medium. Cell morphology, motility and flagellation were investigated using phase-contrast and transmission electron microscopy on cells negatively stained with 0.5 % uranyl acetate. Anaerobic growth was investigated using incubation in the GasPak anaerobic system (BBL) for 15 days at 28 °C on R2A agar. Gram-staining, catalase activity, cytochrome oxidase activity and the hydrolysis of carboxymethylcellulose, casein, chitin, DNA, hypoxanthine, pectin, starch, Tween 80, tyrosine and xanthine were investigated

according to the methods of Smibert & Krieg (1994). Growth at different temperatures and pH values was tested at 5–45 °C (using increments of 5 °C) and pH 4–10 (using increments of 1 pH unit). Salt tolerance was tested on R2A broth supplemented with 0, 1, 2, 3, 5 and 7 % (w/v) NaCl. Flexirubin-type pigments were investigated from observation of a colour-shift response following exposure to a 20 % (w/v) KOH solution (Reichenbach, 1992). Other biochemical tests were carried out by using API 20NE, API ID 32GN and API ZYM test kits according to the manufacturer's instructions (bioMérieux). Phenotypic comparisons between strains JS6-6^T, JS17-8^T and related *Chryseobacterium* species are shown in Table 1.

The 16S rRNA gene was amplified from colonies by a PCR using primers fD1 and rP2 (Weisburg *et al.*, 1991) and the entire PCR fragment was sequenced directly (Hiraishi,

1992). Almost-complete sequences (each approx. 1390 nt) of the 16S rRNA genes of strains JS6-6^T and JS17-8^T were determined. Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed with the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony methods (Fitch, 1971), using the MEGA3 program (Kumar *et al.*, 2004); bootstrap values were based on 1000 replications (Felsenstein, 1985). In the phylogenetic tree, strains JS6-6^T and JS17-8^T are positioned within the genus *Chryseobacterium* (Fig. 1). The sequence similarities between strain JS6-6^T and the type strains of the genus *Chryseobacterium* ranged from 93.7 to 97.1 %. Strain JS6-6^T exhibited 97.1 % sequence similarity with respect to *Chryseobacterium soldanellicola* KCTC 12382^T and *Chryseobacterium piscium* CCUG 51923^T. Strain JS17-8^T showed sequence similarities of 94.0–97.5 % with respect to the type strains of the genus *Chryseobacterium*, exhibiting

Table 1. Phenotypic comparisons of strains JS6-6^T, JS17-8^T and related species

Strains: 1, JS6-6^T; 2, JS17-8^T; 3, *C. joostei* KCTC 12128^T; 4, *C. piscium* CCUG 51923^T; 5, *C. shigense* DSM 17126^T; 6, *C. soldanellicola* KCTC 12382^T. All of the data were obtained in this study. All strains are positive for aesculin hydrolysis, gelatin hydrolysis, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -glucosidase and *N*-acetyl- β -glucosaminidase. All strains are negative for nitrate reduction, glucose fermentation, arginine dihydrolase, β -galactosidase, lipase (C14), α -galactosidase, β -glucuronidase, α -mannosidase and α -fucosidase. All of the strains assimilate D-glucose, D-mannose, maltose and L-proline. None of the strains assimilates capric acid, phenylacetic acid, D-ribose, inositol, itaconic acid, suberic acid, sodium malonate, lactic acid, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, melibiose, L-fucose, D-sorbitol, propionic acid, valeric acid, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid or 4-hydroxybenzoic acid. +, Positive reaction; –, negative reaction.

Characteristic	1	2	3	4	5	6
Isolation source	Soil	Soil	Dairy products	Fresh fish	Lactic acid beverage	Sand-dune plants
Growth on/at:						
MacConkey agar	+	+	+	–	–	–
5 °C	+	+	+	+	+	+
37 °C	–	+	–	–	–	+
Indole production	–	–	–	–	–	+
Urease	+	+	+	+	–	–
Assimilation of:						
<i>N</i> -Acetylglucosamine	–	–	–	–	+	–
Adipic acid	–	–	–	+	+	+
L-Arabinose	–	–	–	+	+	+
Glycogen	+	+	+	–	+	+
Malic acid	–	–	–	+	+	–
D-Mannitol	–	–	–	+	+	–
Potassium gluconate	–	–	–	+	+	+
L-Rhamnose	–	–	–	+	–	+
Salicin	–	–	–	–	+	–
L-Serine	–	–	+	–	+	–
Sodium acetate	–	–	–	+	–	–
Sucrose	+	–	–	–	+	–
Trisodium citrate	–	–	–	+	+	–
Enzymic activities:						
α -Chymotrypsin	–	+	–	–	–	–
Cystine arylamidase	+	+	+	–	–	+
β -Glucosidase	–	–	–	–	+	–
Trypsin	+	+	+	–	+	–

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain JS6-6^T, strain JS17-8^T and related strains. Bootstrap values (based on 1000 replications) below 40 % are not shown. Asterisks indicate that the corresponding branches were also recovered in the maximum-parsimony tree. Bar, 0.005 changes per nucleotide position.

97.5 and 97.4 % similarity to the sequences of *Chryseobacterium joostei* KCTC 12128^T and *Chryseobacterium shigense* DSM 17126^T, respectively.

The following chemotaxonomic characteristics were analysed: isoprenoid quinones (as described by Groth *et al.*, 1996), fatty acids (according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System; Sasser, 1990) and the DNA G+C content (Mesbah *et al.*, 1989). The predominant major respiratory lipoquinone in strains JS6-6^T and JS17-8^T was MK-6. The major fatty acids of strain JS6-6^T included iso-C_{15:0} (15.7 %), iso-C_{17:1ω9c} (15.5 %), iso-C_{17:0} 3-OH (15.0 %) and summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1ω9c}; 12.0 %). The major fatty acids of strain JS17-8^T comprised iso-C_{15:0} (26.8 %), iso-C_{17:0} 3-OH (21.2 %), summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1ω9c}; 12.5 %) and iso-C_{17:1ω9c} (12.1 %) (see Supplementary Table S1, available with the online version of this paper). The DNA G+C contents of strains JS6-6^T and JS17-8^T were 39.9 and 41.4 mol%, respectively.

To determine genomic relatedness, the filter hybridization method was performed as described by Seldin & Dubnau (1985). Probe labelling was conducted by using the non-radioactive DIG-High prime system (Roche); hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA-DNA relatedness was quantified by using a densitometer (Bio-Rad). The DNA-DNA hybridization values for strain JS6-6^T with respect to *C. soldanellicola* KCTC 12382^T and *C. piscium* CCUG 51923^T were 32 and 28 %, respectively. The levels of DNA-DNA relatedness for strain JS17-8^T with respect to *C. joostei* KCTC 12128^T and *C. shigense* DSM 17126^T were 34 and 31 %, respectively.

On the basis of the 16S rRNA gene sequence analysis, strains JS6-6^T and JS17-8^T can be affiliated with the genus *Chryseobacterium*. Both strains can be clearly differentiated from each other and from closely related *Chryseobacterium* species on the basis of isolation source, growth characteristics, enzymic activities and assimilation patterns (Table 1). Furthermore, the fatty acid contents of strain JS6-6^T are distinct because of the presence of moderate amounts of C_{18:1ω7c} (5.7 %) and C_{18:1ω9c} (2.1 %) (see Supplementary Table S1). Thus, strains JS6-6^T and JS17-8^T represent two novel species of the genus *Chryseobacterium*, for which the names *Chryseobacterium soli* sp. nov. and *Chryseobacterium jejuense* sp. nov., respectively, are proposed.

Description of *Chryseobacterium soli* sp. nov.

Chryseobacterium soli (so'li. L. gen. n. *soli* of soil).

Cells are aerobic, Gram-negative, non-motile, non-endo-spore-forming, catalase- and oxidase-positive, straight rods (0.6–0.8 × 1.5–3.0 μm). Yellow colonies with entire edges are formed on R2A agar plates. Flexirubin-type pigments are produced. Growth occurs on R2A, trypticase soy agar, nutrient agar (Difco) and MacConkey agar. Growth occurs at 5–30 °C, the optimum temperature for growth is between 28 and 30 °C. The pH range for growth is 5.0–9.0, with an optimum between pH 6.0 and 7.0. Cells grow in the presence of 0–3 % NaCl, but not with 5 % NaCl. Degrades casein, Tween 80, tyrosine and starch. Chitin, carboxymethylcellulose, DNA, hypoxanthine, pectin and xanthine are not degraded. The predominant cellular fatty acids are iso-C_{15:0}, iso-C_{17:1ω9c}, iso-C_{17:0} 3-OH and summed feature 3. MK-6 is the predominant respiratory quinone. The G+C content of the genomic DNA of the type strain is 39.9 mol%.

The type strain, JS6-6^T (=KACC 12502^T=DSM 19298^T), was isolated from a soil sample from Jeju, Republic of Korea.

Description of *Chryseobacterium jejuense* sp. nov.

Chryseobacterium jejuense (je.ju.en'se. N.L. neut. adj. *jejuense* pertaining to Jeju, Republic of Korea, where the type strain was isolated).

Cells are aerobic, Gram-negative, non-motile, non-endo-spore-forming, catalase- and oxidase-positive, straight rods (0.6–0.7 × 1.0–2.5 µm). Yellow colonies with entire edges are formed on R2A agar plates. Flexirubin-type pigments are produced. Growth occurs on R2A, trypticase soy agar, nutrient agar and MacConkey agar. Growth occurs at 5–35 °C, the optimum temperature for growth is between 28 and 30 °C. The pH range for growth is 5.0–9.0, with an optimum between pH 6.0 and 7.0. Cells grow in the presence of 0–3 % NaCl, but not with 5 % NaCl. Degrades casein, Tween 80, tyrosine and starch. Chitin, carboxymethylcellulose, DNA, hypoxanthine, pectin and xanthine are not degraded. The predominant cellular fatty acids are iso-C_{15:0}, iso-C_{17:0} 3-OH, summed feature 3 and iso-C_{17:1}ω9c. MK-6 is the predominant respiratory quinone. The G + C content of the genomic DNA of the type strain is 41.4 mol%.

The type strain, JS17-8^T (=KACC 12501^T=DSM 19299^T), was isolated from a soil sample from Jeju, Republic of Korea.

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