Chryseobacterium taiwanense sp. nov., isolated from soil in Taiwan

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Among a large collection of Taiwanese soil isolates, a novel Gram-negative, rod-shaped, non-spore-forming, yellow-pigmented bacterial strain, Soil-3-27^T, was isolated from farmland soil in Wu-Feng, Taiwan. The isolate was subjected to a polyphasic study including 16S rRNA gene sequencing, DNA–DNA hybridization, fatty acid analysis and comparative phenotypic characterization. The 16S rRNA gene sequence analysis indicated that the organism belongs to the genus *Chryseobacterium*. The organism contains menaquinone MK-6 as the predominant isoprenoid quinone and 15:0 iso (43%), 17:1 iso ω 9c (17.5%) and 17:0 iso 3-OH (16.6%) as the major fatty acids. Phylogenetically, the closest relatives of strain Soil-3-27^T are *Chryseobacterium daecheongense*, *Chryseobacterium defluvii* and *Chryseobacterium taichungense* with 96.7–97.2% sequence similarity. DNA–DNA hybridization showed relatedness values of 8.5–24.2% with these species. The DNA G + C content is 36.8 mol%. Strain Soil-3-27^T is clearly distinguishable from other *Chryseobacterium* species and represents a novel species, for which the name *Chryseobacterium taiwanense* sp. nov. is proposed. The type strain is strain Soil-3-27^T (=BCRC 17412^T=IAM 15317^T=LMG 23355^T).

The genus *Chryseobacterium*, a member of the family *Flavobacteriaceae* initially proposed to group several bacterial species previously attributed to the genus *Flavobacterium* (Vandamme *et al.*, 1994), currently consists of 13 species with *Chryseobacterium gleum* as the type species. Two additional species were recently transferred to the new genus *Elizabethkingia* (Kim *et al.*, 2005). The name of the protein-deaminating taxon '*Chryseobacterium proteolyticum*' (Yamaguchi & Yokoe, 2000) was not validly published under the Rules of the *Bacteriological Code* (1990 Revision) (Lapage *et al.*, 1992).

In the present study, the taxonomic position of a Taiwanese soil isolate was studied using a polyphasic approach. Based on 16S rRNA gene sequence similarity, DNA–DNA relatedness values, fatty acid content and phenotypic characterization, we propose the name *Chryseobacterium taiwanense* sp. nov. for this novel species.

Strain Soil-3-27^T was isolated on Luria–Bertani agar (USB) from a sample of farmland soil from Taiwan. The type

strains of *Chryseobacterium* and *Elizabethkingia* species were received from culture collections. All strains were reactivated on trypticase soy agar (Difco) and checked for purity on nutrient agar (Difco) after cultivation at 30 °C for 24–48 h. Strains were preserved at –80 °C in trypticase soy broth (Difco) with 10% (v/v) glycerol or by lyophilization. The patent strain '*C. proteolyticum*' FERM P-17664 was included in the study using data from the NCBI database and literature (Yamaguchi & Yokoe, 2000).

Genomic DNA was extracted and purified using the Qiagen Blood and Cell Culture DNA kit. The 16S rRNA genes were PCR-amplified and sequenced using the MicroSeq Full Gene 16S rDNA Bacterial Sequencing kit (PE Applied Biosystems). Sequencing was performed using an Applied Biosystem 310 DNA sequencer (PE Applied Biosystems). Sequence assembly was performed using the ABI Prism DNA Sequencing Analysis software (PE Applied Biosystems) and phylogenetic analyses were performed using the BioNumerics software (version 3.1; Applied Maths: <http://www.applied-maths.com/>), CLUSTAL_X (Thompson *et al.*, 1997) and MEGA version 3.1 (Kumar *et al.*, 2004). The consensus sequence of strain Soil-3-27^T was aligned with those of *Chryseobacterium* and *Elizabethkingia* species retrieved from the NCBI database.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BCRC 17412^T is DQ318789.

Sequences of *Sejongia antarctica* IMSNU 14040^T, *Kaistella koreensis* IAM 15050^T, *Bergeyella zoohelcum* ATCC 43767^T, *Flavobacterium aquatile* ATCC 11947^T, *Riemerella anatipestifer* ATCC 11845^T, *Ornithobacterium rhinotracheale* LMG 9086^T, *Weeksella virosa* ATCC 43766^T and *Empedobacter brevis* ATCC 14234 were also included in the phylogenetic analysis for comparative purpose. Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. The confidence values of branches on the neighbour-joining tree were determined by performing a bootstrap analysis with 1000 replicates.

DNA G + C content and DNA–DNA relatedness values were determined with genomic DNA prepared using a commercial kit (Genomic-tips; Qiagen). The DNA G + C content was determined using reversed-phase HPLC according to Tamaoka & Komagata (1984) with slight modifications. Nucleotides were separated using a Cosmosil 5C₁₈ column (4.0 × 150 mm) (Waters) in a mobile phase composed of 0.2 M NH₄H₂PO₄/acetonitrile (20:1, v/v) at a flow rate of 1 ml min⁻¹ at room temperature. Nucleotides were detected and quantified by absorption at 260 nm. DNA relatedness values between the novel strain and the type strains of *Chryseobacterium* and *Elizabethkingia* species were determined using the fluorometric hybridization method in microdilution wells (Ezaki *et al.*, 1989; Chern *et al.*, 2004). The fluorescence intensity of each well was measured with a Fluoroskan II microplate fluorometer (Labsystems) at a wavelength of 360 nm for excitation and at 450 nm for emission. Hybridization temperature was 40 °C.

The fatty acid content was determined using the Sherlock Microbial Identification system (MIDI) as described previously (Chern *et al.*, 2004). Extracts of the methylated fatty acids were prepared according to the protocol provided by the manufacturer and analysed with Agilent 6890N gas chromatograph equipped with a flame-ionization detector and 7683 Automatic Liquid Sampler (Agilent). Identification of the peaks was made by comparing the results with the built-in TSBA 50 database (MIDI).

Respiratory quinones of the novel bacterium were determined according to Collins & Jones (1981) and Komagata & Suzuki (1987). The TLC-purified quinones were analysed with a Nova-Pak C₁₈ (15 × 3.9 cm) column (Waters). Peaks were detected at 270 nm after elution with methanol/2-propanol (2:1) at flow rate of 1 ml min⁻¹.

All phenotypic tests were performed on bacteria grown on nutrient agar at 30 °C unless otherwise specified. General cell morphology was studied using phase-contrast microscopy (Eclipse E600; Nikon). Gram reaction was performed using a 4-Step Gram Stain kit (Becton Dickinson) according to the manufacturer's instructions. Catalase activity, oxidase activity and oxidation–fermentation reactions were determined using standard methods (Barrow & Feltham, 1993). Flexirubin-type pigments were detected according to Li *et al.*

(2003). Additional physiological and biochemical tests were performed using GN2 MicroPlate (Biolog), API 20E and API ZYM (bioMérieux) according to the manufacturer's instructions. A battery of tests was selected to differentiate among Gram-negative, yellow-pigmented bacteria (Holmes *et al.*, 1984). Tests were performed at 30 °C with different incubation times, as described by Cowan (1974), MacFaddin (1980) or Gerhardt *et al.* (1981), unless indicated otherwise.

The 16S rRNA gene sequence analysis showed that strain Soil-3-27^T belongs to the genus *Chryseobacterium* (Fig. 1). Although sequence similarities of 96.7–97.2 % between strain Soil-3-27^T and the type strains of *C. daecheongense*, *C. defluvii* and *C. taichungense* did not exclude a possible relationship at the species level with one of these taxa

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between *Chryseobacterium* species and related taxa in the family *Flavobacteriaceae*. The GenBank accession numbers for the sequences used in the analysis are given in parentheses. Bootstrap percentages (based on 1000 replicates) are given at the branching points. *Flavobacterium aquatile* ATCC 11947^T, *Riemerella anatipestifer* ATCC 11845^T, *Weeksella virosa* ATCC 43766^T and *Empedobacter brevis* ATCC 14234 were used as the outgroup to root the tree. Bar, 0.02 substitutions per nucleotide position.

Table 1. Fatty acid content of *Chryseobacterium* and *Elizabethkingia* species

Species: 1, *C. taiwanense* sp. nov.; 2, *C. defluvi*; 3, *C. daecheongense*; 4, *C. taichungense*; 5, *C. joostei*; 6, *C. vrystaatense*; 7, *C. gleum*; 8, *C. indologenes*; 9, *C. formosense*; 10, *C. indoltheticum*; 11, *C. balustinum*; 12, *C. scopthalmum*; 13, *E. meningoseptica*; 14, *E. miricola*. Data from Shen *et al.* (2005); Young *et al.* (2005); de Beer *et al.* (2005) and Kim *et al.* (2005). Values given are mean $\pm$ SD. tr, Trace (less than 1.0%); ND, not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14
13:0 iso	1.0 $\pm$ 0.1	4.0 $\pm$ 0.1	1.3 $\pm$ 0.1	tr	1.3	1.1 $\pm$ 0.4	tr	tr	3.6	tr	1.7 $\pm$ 0.1	tr	1.3 $\pm$ 0.3	2.0 $\pm$ 0.5
unknown 13:565*	2.6 $\pm$ 0.1	2.9 $\pm$ 0.1	1.4 $\pm$ 0.4	6.4	2.0 $\pm$ 0.1	1.4 $\pm$ 0.3	2.7 $\pm$ 0.5	2.2 $\pm$ 0.2	tr	2.2 $\pm$ 0.3	1.9 $\pm$ 0.2	3.4 $\pm$ 1.0	1.9 $\pm$ 0.3	1.5 $\pm$ 0.2
15:0 iso	43.2 $\pm$ 0.7	53.2 $\pm$ 0.5	46.8 $\pm$ 2.1	35.4	39.6 $\pm$ 0.3	41.8 $\pm$ 1.4	34.4 $\pm$ 0.2	33.0 $\pm$ 0.4	52.2	32.6 $\pm$ 0.5	35.4 $\pm$ 0.3	37.4 $\pm$ 0.7	43.9 $\pm$ 2.0	46.4 $\pm$ 2.2
15:0 anteiso	1.0 $\pm$ 0.1	1.9 $\pm$ 0.1	1	tr	tr	1.7 $\pm$ 0.7	tr	tr	3.2	6.2	1.1	tr	1.1 $\pm$ 0.8	1.0 $\pm$ 0.6
16:0	1.4 $\pm$ 0.1	1.5 $\pm$ 0.1	1.4	1.3	1.7	1.1 $\pm$ 0.3	1.8 $\pm$ 0.1	2.1 $\pm$ 0.1	1.5	2.0 $\pm$ 0.2	3.5 $\pm$ 0.1	1.6 $\pm$ 0.1	tr	1.2 $\pm$ 0.1
15:0 iso 3-OH	3.8	2.5	2.8 $\pm$ 0.1	4.3	2.7	2.7 $\pm$ 0.3	2.9	2.8	1.8	2.2	2.5	3.0 $\pm$ 0.1	2.8 $\pm$ 0.3	3.0 $\pm$ 0.6
17:1 iso ω 9c	17.5 $\pm$ 0.2	60.0 $\pm$ 0.2	10.8 $\pm$ 0.7	8.9	19.5 $\pm$ 0.1	19.7 $\pm$ 2.3	21.0 $\pm$ 0.5	22.2 $\pm$ 0.2	4.3	20.2 $\pm$ 0.4	24.5 $\pm$ 0.6	22.9 $\pm$ 0.2	7.8 $\pm$ 1.3	6.6 $\pm$ 0.2
unknown 16:582*	0.8 $\pm$ 0.2	tr	1.4 $\pm$ 0.1	1.7	1.0	1.2 $\pm$ 0.2	1.1	1.1	1.0	tr	tr	tr	1.6 $\pm$ 0.1	1.3 $\pm$ 0.6
17:0 iso	1.3 $\pm$ 0.1	2.6 $\pm$ 0.1	tr	tr	2.4	Tr	2.3	1.6 $\pm$ 0.1	2.3	1.6 $\pm$ 0.2	3.1	1.5 $\pm$ 0.1	tr	tr
16:0 3-OH	tr	tr	tr	2.6	1.0	1.3 $\pm$ 0.3	1.2	1.2	tr	1.2 $\pm$ 0.1	1.3	1.1 $\pm$ 0.1	2.6 $\pm$ 0.4	3.0 $\pm$ 0.6
17:0 iso 3-OH	16.6 $\pm$ 1.1	15.6 $\pm$ 0.5	20.5 $\pm$ 1.6	22.4	17.9 $\pm$ 0.1	15.4 $\pm$ 1.8	19.3 $\pm$ 0.8	18.5 $\pm$ 0.3	10.9	14.5 $\pm$ 0.5	15.2 $\pm$ 0.4	15.9 $\pm$ 1.7	14.6 $\pm$ 1.0	15.3 $\pm$ 0.2
17:0 2-OH	tr	ND	tr	ND	ND	ND	ND	ND	ND	3.0 $\pm$ 0.3	tr	tr	ND	ND
Summed feature 3†	7.9 $\pm$ 0.1	7.4 $\pm$ 0.2	8.8 $\pm$ 0.2	ND	8.4 $\pm$ 0.1	9.1 $\pm$ 0.9	10.4 $\pm$ 0.1	11.0 $\pm$ 0.2	ND	9.4 $\pm$ 0.1	5.6	8.3 $\pm$ 0.3	ND	ND
Summed feature 4†	ND	ND	ND	13.8	ND	tr	ND	ND	6.5	ND	ND	ND	19.6 $\pm$ 1.0	17.0 $\pm$ 1.3
Summed feature 5†	tr	ND	tr	ND	tr	ND	tr	1.2	ND	ND	ND	tr	tr	ND

*Unknown fatty acid; number indicates equivalent chain-length.

†Fatty acids that could not be separated by gas chromatography using the Microbial Identification system (Microbial ID) software were considered summed features. Summed feature 3 contains 15:0 iso 2-OH and/or 16:1 ω 7c. Summed feature 4 contains 15:0 iso 2-OH and/or 16:1 ω 7c/t. Summed feature 5 contains 17:1 iso I and/or 17:1 anteiso B.

(Vandamme *et al.*, 1996), DNA–DNA relatedness values between the four strains were only 8.5–24.2 %. All other reference strains studied shared lower 16S rRNA gene sequence similarity and insignificant DNA relatedness with strain Soil-3-27^T. Therefore, the novel isolate represents a novel species in the genus *Chryseobacterium*.

Fatty acid content of strain Soil-3-27^T and related taxa (except '*C. proteolyticum*' for which no data are available) are presented in Table 1. Only minor differences between strain Soil-3-27^T and other *Chryseobacterium* and *Elizabethkingia* species were noted. Dominant fatty acids for all strains investigated (mean level above 15 %) were 15:0 iso, 17:0 iso 3-OH and 17:1 iso ω 9c (Table 1). Strain Soil-3-27^T

contained menaquinone MK-6 as the predominant isoprenoid quinone; this is in accordance with all other members of the family *Flavobacteriaceae*.

Phenotypic features that distinguish the novel species from other *Chryseobacterium* and *Elizabethkingia* species are listed in Table 2 and in the species description.

Description of *Chryseobacterium taiwanense* sp. nov.

Chryseobacterium taiwanense (tai.wan.en'se. N.L. neut. adj. *taiwanense*, pertaining to Taiwan, the location of the soil sample from which the type strain was isolated).

Table 2. Differential phenotypic characteristics of *Chryseobacterium* and *Elizabethkingia* species

Species: 1, *C. taiwanense* sp. nov.; 2, *C. defluvii*; 3, *C. daecheongense*; 4, *C. taichungense*; 5, *C. joostei*; 6, *C. vrystaatense*; 7, *C. shigense*; 8, *C. gleum*; 9, *C. indologenes*; 10, *C. formosense*; 11, *C. indoltheticum*; 12, *C. balustinum*; 13, *C. scophthalmum*; 14, *E. meningoseptica*; 15, *E. miricola*. Data from Shen *et al.* (2005); Young *et al.* (2005); de Beer *et al.* (2005); Shimomura *et al.* (2005) and Kim *et al.* (2005). Only the type strain of each species was included. +, Positive; –, negative; W, weak; V, variable; NA, no data available. All strains tested are negative for nitrate reduction.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Growth at (°C):															
5	+	+	–	–	+	+	+	–	–	–	+	+	+	–	–
37	+	+	+	+	+	–	–	+	+	–	–	–	–	+	+
42	+	+	–	–	+	–	NA	+	–	–	–	–	–	–	+
Acid production from:															
L-Arabinose	+	V	–	–	–	NA	–	+	–	–	–	–	–	–	–
D-Arabinose	–	–	–	NA	–	NA	NA	–	–	–	–	–	–	NA	NA
D-Cellobiose	+	V	+	NA	–	NA	NA	V	–	–	+	+	+	–	–
D-Fructose	V	–	+	NA	+	NA	+	+	–	NA	–	+	+	+	+
Trehalose	–	+	+	W	+	+	–	+	+	+	–	V	+	+	+
Lactose	–	–	–	–	–	NA	–	–	–	NA	–	–	–	+	+
Sucrose	–	–	–	–	–	NA	NA	–	–	–	–	+	–	–	–
Mannitol	–	–	–	–	–	V	–	–	–	–	–	–	–	+	+
Glycerol	–	–	+	NA	+	NA	–	+	V	–	–	–	–	+	V
Urease activity	–	–	–	–	+	+	–	+	–	–	–	+	+	+	+
Nitrite reduction	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
Production of indole	+	–	–	W	+	+	+	+	+	+	+	+	+	+	V
Production of H ₂ S	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
Utilization of citrate	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+
Hydrolysis of:															
2-Naphthyl butyrate	–	W	–	+	–	NA	NA	–	–	+	–	–	–	–	+
L-Cystyl 2-naphthylamide	–	–	–	+	–	NA	NA	–	–	+	–	–	+	+	+
N-Benzoyl-DL-arginine 2-naphthylamide	–	+	–	+	+	NA	NA	–	–	+	–	–	+	+	+
N-Glutaryl-phenylalanine 2-naphthylamide	–	–	+	+	–	NA	NA	–	+	–	–	–	–	+	–
6-Bromo-2-naphthyl α -D-galactopyranoside	–	–	–	–	–	NA	NA	–	–	–	–	–	–	+	–
2-Naphthyl β -D-galactopyranoside	–	–	–	W	–	NA	NA	–	–	–	–	–	–	–	+
2-Naphthyl α -D-glucopyranoside	+	+	–	+	+	NA	NA	+	+	–	+	–	–	+	+
6-Bromo-2-naphthyl β -D-glucopyranoside	–	–	–	+	–	NA	NA	+	–	+	–	–	–	–	–
1-Naphthyl N-acetyl- β -D-glucosaminide	–	+	+	+	+	NA	NA	+	+	+	+	+	+	+	+
2-Naphthyl α -L-fucopyranoside	–	–	–	–	–	NA	NA	–	–	–	–	–	–	+	+
Starch	+	+	+	NA	+	–	+	+	+	NA	–	–	–	–	–
Tween 80	+	+	+	V	+	+	NA	+	+	–	+	+	+	–	–

Gram-negative, non-spore-forming and non-motile rods 1.06–2.24 × 0.58–0.85 µm in size. Colonies are yellowish, translucent and shiny with entire edges. Growth occurs at 5–42 °C (optimum 30 °C) on trypticase soy agar. Growth does not occur below 5 °C or above 42 °C. The pH range for growth is 5–10 (optimum 6–8). Growth occurs on trypticase soy agar containing 4 % NaCl (w/v). Catalase and oxidase-positive, urease activity is absent. Produces flexirubin-type pigments. Indole is produced. Tween 80, casein, aesculin, gelatin and starch are hydrolysed. Acid is produced from α -cyclodextrin, D-psicose, D-raffinose, glucuronamide, glycyl-L-glutamic acid, γ -aminobutyric acid, inosine, uridine, thymidine and glycerol. No acid is produced from any other substrate in GN2 MicroPlate. The following substrates are degraded in API ZYM strip: 2-naphthyl phosphate (at pH 8.5 and 5.4), 2-naphthyl caprylate, L-leucyl 2-naphthylamide, L-valyl 2-naphthylamide, naphthol-AS-BI phosphate and 2-naphthyl α -D-glucopyranoside. The following substrates are not degraded in API ZYM strip: 2-naphthyl butyrate, 2-naphthyl myristate, L-cystyl 2-naphthylamide, N-benzoyl-DL-arginine 2-naphthylamide, N-glutaryl-phenylalanine 2-naphthylamide, 6-bromo-2-naphthyl α -D-galactopyranoside, 2-naphthyl β -D-galactopyranoside, naphthol-AS-BI β -D-glucuronide, 6-bromo-2-naphthyl β -D-glucopyranoside, 1-naphthyl-N-acetyl β -D-glucosaminide, 6-bromo-2-naphthyl α -D-mannopyranoside or 2-naphthyl α -L-fucopyranoside. The detailed fatty acid content and additional phenotypic features are given in Tables 1 and 2, respectively. Menaquinone MK-6 is the predominant respiratory quinone. Major fatty acids are 15:0 iso (43 %), 17:1 iso ω 9c (17.5 %) and 17:0 iso 3-OH (16.6 %). The G+C content of the DNA is 36.8 mol%.

The type strain is strain Soil-3-27^T (= BCRC 17412^T = IAM 15317^T = LMG 23355^T), isolated in the year 2000 from farmland soil in Wu-Feng, Taiwan.

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