Chryseobacterium taichungense sp. nov., isolated from contaminated soil

Fo-Ting Shen,¹ Peter Kämpfer,² Chiu-Chung Young,¹ Wei-An Lai¹ and A. B. Arun¹

Correspondence

Peter Kämpfer
peter.kaempfer@agrar.
uni-giessen.de

¹College of Agriculture and Natural Resources, Department of Soil and Environmental Sciences, National Chung Hsing University, Taichung 402, Taiwan, Republic of China

²Institut für Angewandte Mikrobiologie, Justus-Liebig Universität Giessen, Heinrich-Buff-Ring 26–32 (IFZ), D-35392 Giessen, Germany

A bacterial strain (CC-TWGS1-8^T) isolated from a tar-contaminated soil in Taiwan was studied in a detailed taxonomic study. The cells were Gram-negative, rod-shaped and non-spore-forming. Phylogenetic analyses using the 16S rRNA gene sequence of the strain clearly revealed an affiliation to the genus *Chryseobacterium*, the highest sequence similarities being to the type strain of *Chryseobacterium indologenes* (96.8%), to *Chryseobacterium gleum* (96.8%) and to *Chryseobacterium joostei* (96.4%). The 16S rRNA gene sequence similarities to all other *Chryseobacterium* species were below 96%. The major whole-cell fatty acids were 15:0 iso (35.4%) and 17:0 iso 3OH (22.5%). DNA–DNA hybridization values and the biochemical and chemotaxonomic properties demonstrate that strain CC-TWGS1-8^T represents a novel species, for which the name *Chryseobacterium taichungense* sp. nov. is proposed. The type strain is CC-TWGS1-8^T (= CCUG 50001^T = CIP 108519^T).

At present, the genus *Chryseobacterium* contains 12 species: *Chryseobacterium balustinum*, *Chryseobacterium gleum*, *Chryseobacterium indologenes*, *Chryseobacterium indoltheticum*, *Chryseobacterium meningosepticum*, *Chryseobacterium miricola*, *Chryseobacterium 'proteolyticum'* (name not validated), *Chryseobacterium scophthalmum*, *Chryseobacterium joostei*, *Chryseobacterium defluvii*, *Chryseobacterium daecheongense* and *Chryseobacterium formosense*. These species and their taxonomy have been extensively described in recently published reports (Hugo *et al.*, 2003; Li *et al.*, 2003; Kämpfer *et al.*, 2003; Young *et al.*, 2005).

A yellow-pigmented isolate from a tar-contaminated soil from Taichung, Taiwan, was obtained on nutrient agar. This strain (CC-TWGS1-8^T) was maintained and subcultured on nutrient agar (Oxoid) at 30 °C for 48 h and subsequently analysed to determine the 16S rRNA gene sequence, the fatty acid methyl ester composition of whole-cell hydrolysates, additional phenotypic characteristics, and DNA–DNA relatedness to those species most closely related on the basis of 16S rRNA similarity.

Cultural and morphological characteristics were observed on nutrient agar. The Gram reaction was tested by using the modified method of Gerhardt *et al.* (1994), while motility

was examined microscopically from cells grown for 3 days in motility broth (Cowan, 1974) at 30 °C.

Strain CC-TWGS1-8^T was Gram-negative and formed visible (about 2 mm) yellowish colonies in 48 h at 30 °C. No growth was observed below 10 °C or above 37 °C. At 15–36 °C, clearly visible colonies appeared within 48 h. The colonies were translucent and shiny with entire edges, but upon prolonged incubation the colonies were not visible as single entities, probably because of the profuse production of extracellular substances. A bright-yellow pigment (flexirubin) was produced on nutrient agar. Oxidase activity was tested using oxidase reagent (bioMérieux) according to the instructions of the manufacturer. Strain CC-TWGS1-8^T was oxidase-positive. Cells were non-motile, non-spore-forming rods (1 µm wide by 2 µm long). CC-TWGS1-8^T was able to grow well on nutrient agar, brain heart infusion agar (Oxoid) and trypticase soy agar (Oxoid) but was unable to grow on MacConkey agar (Oxoid).

Physiological characterization and additional biochemical tests were performed to assess the carbon-source utilization pattern, while the hydrolysis of 19 substrates was investigated as described by Kämpfer *et al.* (1991). Additional biochemical tests were performed using the Micronaut-E system (formerly called the Titertek-Enterobac-Automated System; Kämpfer, 1990). Growth was also investigated at different temperatures (4, 11, 30, 37 and 45 °C) in nutrient broth. The API ZYM system (bioMérieux) was used to test for the presence of 19 constitutive enzymes.

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The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain CC-TWGS1-8^T is AJ843132.

The cellular fatty acid composition (determined using the method described by Kämpfer & Kroppenstedt, 1996) showed that 15:0 iso was the most abundant fatty acid (35.4%), followed by 17:0 iso 3OH (22.5%) and summed feature 4 (15:0 iso 2OH/16:1 ω 7t, 13.8%). Table 1 shows the fatty acid pattern for strain CC-TWGS1-8^T in comparison with those of all other *Chryseobacterium* species.

The 16S rRNA gene was analysed as described by Kämpfer *et al.* (2003). A phylogenetic analysis (Fig. 1) was performed using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar *et al.*, 2001) after multiple sequence alignment of data by using CLUSTAL_X (Thompson *et al.*, 1997). Distances (using distance options according to the Kimura two-parameter model) and clustering using the neighbour-joining method and maximum parsimony (all according to Kumar *et al.*, 2001) were determined by using bootstrap values based on 1000 replications. Strain CC-TWGS1-8^T was phylogenetically most closely related to species of the genus *Chryseobacterium*. According to the sequence similarity calculations, the most closely related strains were the type strains of *C. indologenes* and *C. gleum*, both of which showed 96.8% similarity to strain CC-TWGS1-8^T. Furthermore, the type strain of *C. joostei* showed 96.4% 16S rRNA sequence similarity to CC-TWGS1-8^T, whereas sequence similarities were below 96.0% for all other *Chryseobacterium* species.

DNA–DNA hybridization experiments were performed with CC-TWGS1-8^T and type strains of the three most closely related *Chryseobacterium* species, using a method described by Ziemke *et al.* (1998) (except that for nick translation, 2 μ g DNA was labelled during a 3 h incubation at 15 °C). Strain CC-TWGS1-8^T showed relatively low DNA–DNA hybridization to the type strains of *C. indologenes* (LMG 8337^T; 34.1%, reciprocal 25.1%), *C. gleum* (LMG 8334^T; 43.4%, reciprocal 35.1%) and *C. joostei* (CIP 105533^T; 10.7%, reciprocal 21.1%).

Strain CC-TWGS1-8^T utilized only a few carbon sources but was able to hydrolyse many chromogenic substrates. The results of biochemical/physiological tests are given in Table 2 and in the species description.

On the basis of the results of this polyphasic study, it is clear that strain CC-TWGS1-8^T represents a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium taichungense* sp. nov. is proposed.

Description of *Chryseobacterium taichungense* sp. nov.

Chryseobacterium taichungense (tai.chung'en.se. N.L. neut. adj. *taichungense* pertaining to Taichung, a province in Taiwan).

Table 1. Long-chain fatty acid composition of *Chryseobacterium* species and related bacteria

Taxa: 1, *C. taichungense* sp. nov. ($n=1$); 2, *C. formosense* ($n=1$); 3, *C. defluvii* ($n=1$); 4, *C. joostei* ($n=11$); 5, *C. gleum* ($n=5$); 6, *C. indologenes* ($n=45$); 7, *C. balustinum* ($n=1$); 8, *C. indoltheticum* ($n=1$); 9, *C. scophthalmum* ($n=2$); 10, *C. meningosepticum* ($n=1$); 11, *C. miricola* ($n=1$); 12, *Bergeyella zoohelcum* ($n=1$); 13, *Empedobacter brevis* ($n=6$). Fatty acid percentages amounting to less than 1% of the total fatty acids in all strains were not included. Means $\pm$ SD are given. Tr, Trace (less than 1.0%); ND, not detected. Data are from Hugo *et al.* (2003), Kämpfer *et al.* (2003), Li *et al.* (2003) and Young *et al.* (2005).

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13
13:0 iso	Tr	3.6	2.8	Tr	Tr	Tr	Tr	ND	Tr	1.4	Tr	1.5	Tr
Unknown 13:566*	6.7	Tr	Tr	1.1 $\pm$ 0.2	1.2 $\pm$ 0.4	2.1 $\pm$ 0.7	1.6	1.7	2.9 $\pm$ 0.2	1.5	Tr	1.8	1.9 $\pm$ 1.4
15:0 iso	35.4	52.2	58.5	34.6 $\pm$ 2.0	35.4 $\pm$ 2.9	34.3 $\pm$ 4.9	32.3	29.4	35.0 $\pm$ 0.7	41.4	33.0	47.8	23.6 $\pm$ 2.2
15:0 iso 3OH	4.3	1.8	2.6	2.9 $\pm$ 0.3	2.5 $\pm$ 0.1	2.6 $\pm$ 0.2	2.7	2.3	2.7 $\pm$ 0.1	3.5	3	3.95	4.8 $\pm$ 0.7
15:0 anteiso	0.7	2.1	3.2	Tr	Tr	Tr	Tr	5.9	Tr	2.3	Tr	ND	Tr
16:0	1.3	1.5	1.3	Tr	1.3 $\pm$ 0.4	Tr	1.6	1.0	1.2 $\pm$ 0.2	Tr	2	Tr	3.8 $\pm$ 0.4
16:0 3OH	2.6	Tr	Tr	1.2 $\pm$ 0.2	1.1 $\pm$ 0.1	1.0 $\pm$ 0.2	1.4	Tr	1.0 $\pm$ 0.1	2.2	8	ND	3.6 $\pm$ 0.7
16:0 iso 3OH	1.4	1.1	Tr	ND	ND	ND	Tr	1.3	ND	Tr	Tr	ND	Tr
Unknown 16:580*	1.7	1.0	ND	1.6 $\pm$ 0.1	1.7 $\pm$ 0.1	1.7 $\pm$ 0.2	1.3	1.3	1.5 $\pm$ 0.1	1.7	Tr	1.4	1.4 $\pm$ 0.1
17:0 2OH	ND	ND	ND	ND	ND	ND	Tr	3.0	Tr	Tr	ND	ND	Tr
17:0 iso	0.8	2.3	2.0	Tr	1.6 $\pm$ 0.6	Tr	1.0	Tr	Tr	Tr	1	ND	Tr
17:0 iso 3OH	22.4	10.9	14.1	20.1 $\pm$ 1.2	21.8 $\pm$ 0.3	19.2 $\pm$ 1.8	16.8	14.0	16.3 $\pm$ 0.1	16.3	40	13.5	17.1 $\pm$ 2.1
17:1 iso ω 9c	8.9	4.3	4.8	22.9 $\pm$ 1.9	20.2 $\pm$ 3.9	24.2 $\pm$ 3.1	27.1	25.6	24.8 $\pm$ 0.4	7.0	Tr	17.5	3.9 $\pm$ 0.5
18:1 ω 5c	ND	ND	ND	Tr	Tr	Tr	Tr	Tr	Tr	Tr	ND	1.5	ND
Summed feature 4†	13.8	6.5	8.4	12.1 $\pm$ 1.3	11.8 $\pm$ 0.8	11.1 $\pm$ 1.3	9.2	11.2	11.5 $\pm$ 0.3	18.1	12	9.0	19.4 $\pm$ 1.6
Summed feature 5†	ND	ND	ND	Tr	Tr	Tr	Tr	ND	Tr	Tr	Tr	1.5	6.7 $\pm$ 0.8

*Unknown fatty acid; numbers indicate equivalent chain length.

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

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the European Molecular Biology Laboratory database (accession numbers are given in parentheses), constructed after multiple alignment of data by using CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining method were determined by using the software packages MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar *et al.*, 2001). Bootstrap values, based on 1000 replications, are given as percentages at the branching points. Bar, 0.02 nt substitution (K_{nuc}) unit.

Table 2. Comparison of characteristics of strain CC-TWGS1-8^T with those of other reported *Chryseobacterium* species

Species: 1, *C. taichungense*; 2, *C. formosense*; 3, *C. defluvii*; 3, 4, *C. 'proteolyticum'* ($n=2$); 5, *C. gleum* ($n=12$); 6, *C. indologenes* ($n=13$); 7, *C. balustinum* ($n=1$); 8, *C. indoltheticum* ($n=1$); 9, *C. meningosepticum* ($n=49$); 10, *C. scophthalmum* ($n=7$); 11, *C. joostei* ($n=11$); 12, *C. miricola* ($n=1$). Data for reference species were taken from Kämpfer *et al.* (2003), Li *et al.* (2003), Hugo *et al.* (2003) and Young *et al.* (2005). n , Number of strains tested; +, all strains tested positive; (+), weakly positive; –, all strains tested negative; NA, not available. Two figures separated by a solidus (/) refer to the number of positive strains out of the number of strains tested.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Acid production from:*												
Glucose	(+)	(+)	+	+	+	+	+	+	+	–	+	+
Sucrose	–	–	–	+	–	–	–	–	–	–	–	–
Mannitol	–	–	–	+	–	4/13	–	–	31/49	–	+	+
Arabinose	–	–	–	+	10/12	–	–	–	1/49	–	–	–
Cellobiose	–	–	(+)	–	–	–	–	–	4/49	–	–	–
Fructose	NA	NA	NA	NA	+	+	+	–	28/49	–	+	+
Lactose	–	NA	NA	–	–	–	–	–	27/49	–	–	+
Maltose	+	–	+	+	+	+	–	+	46/49	–	+	+
Trehalose	+	+	+	+	+	+	–	–	42/49	–	+	+
Xylose	+	(+)	–	NA	4/12	–	–	–	3/49	–	–	–
Casein digestion	NA	NA	NA	+	+	NA	+	+	+	–	+	NA
Aesculin hydrolysis	+	+	+	+	+	+	–	+	47/49	–	+	+
Growth at 36–37 °C	+	–	+	+	+	+	–	+	+	–	–	+
Growth on MacConkey agar	–	–	–	–	+	V	+	+	+	–	+	(+)
Nitrate reduction	–	–	–	–	7/12	V	+	–	–	–	–	–
Urease activity	–	–	–	–	7/12	–	–	–	18/49	+	+	+
Indole production	(+)	+	+	+	+	+	+	+	24/49	–	+	+
Malonate utilization	–	–	–	–	NA	+	NA	NA	NA	NA	–	NA

*Different methods were used for testing acid formation from different sugars; the original reports should be consulted for direct comparisons.

Cells are Gram-negative, non-motile, non-spore-forming rods (approx. 2 µm in length). Aerobic, oxidase-positive, shows good growth after 48 h on nutrient agar, brain heart infusion agar and trypticase soy agar at 11–36 °C, but unable to grow on MacConkey agar. Colonies on nutrient agar are smooth, yellowish, circular, translucent and shiny with entire edges, becoming mucoid and unidentifiable as single entities after prolonged incubation. The yellow pigmentation is non-diffusible and non-fluorescent. Unable to grow at 4 and 45 °C. Positive for gelatinase activity. Grows well over a broad range of pH values (6.0–9.0) but grows better in neutral or weakly alkaline conditions (pH 7.0–8.0). Major cellular fatty acids are 15:0 iso and 17:0 iso 3OH. Positive reactions observed for alkaline phosphatase, butyrate esterase, carprylate esterase, myristate lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -glucuronidase, α -glucosidase, β -glucosidase and *N*-acetyl- β -glucosaminidase. No enzymic activities found for α -galactosidase, α -mannosidase or α -fucosidase. Weakly positive for indole production, cytochrome oxidase activity and β -galactosidase (ONPG test), but negative for arginine dihydrolase, lysine decarboxylase, citrate utilization, H₂S production, urease and tryptophan deaminase. Strain CC-TWGS1-8^T shows weak production of acid from adonitol, D-glucose, i-inositol, D-maltose, L-rhamnose, D-trehalose and D-xylose. No acid is produced from L-arabinose, D-arabitol, dulcitol, erythritol, lactose, D-mannitol, D-melibiose, methyl α -D-glucoside, raffinose, salicin, D-sorbitol or sucrose. The following compounds are not utilized as sole sources of carbon: D-glucose, D-maltose, D-mannose, D-trehalose, acetate, propionate, *N*-acetylglucosamine, *N*-acetylglucosamine, L-arabinose, L-arbutin, D-cellobiose, D-galactose, gluconate, glycerol, D-fructose, D-mannitol, maltitol, α -D-melibiose, L-rhamnose, D-ribose, D-sucrose, salicin, D-trehalose, D-xylose, adonitol, i-inositol, D-sorbitol, putrescine, *cis*-aconitate, *trans*-aconitate, 4-aminobutyrate, adipate, azelate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, 2-oxoglutarate, pyruvate, suberate, citrate, mesaconate, L-alanine, β -alanine, L-ornithine, L-phenylalanine, L-serine, L-aspartate, L-histidine, L-leucine, L-proline, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate. The chromogenic substrates *p*-nitrophenyl- α -D-glucopyranoside, *p*-nitrophenyl- β -D-glucopyranoside, *p*-nitrophenyl- β -D-galactopyranoside and *p*-nitrophenyl- β -D-xylopyranoside, bis(*p*-nitrophenyl) phosphate, bis(*p*-nitrophenyl) phenylphosphonate, bis-*p*-nitrophenylphosphorylcholine, 2-deoxythymidine 2'-*p*-nitrophenyl-phosphate, L-alanine-*p*-nitroanilide, γ -L-glutamate-*p*-nitroanilide and L-proline-*p*-nitroanilide are hydrolysed. *p*-Nitrophenyl β -D-glucuronide is not hydrolysed.

The type strain is CC-TWGS1-8^T (=CCUG 50001^T=CIP 108519^T), which was isolated from tar-contaminated soil in Taichung, Taiwan.

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