Chryseobacterium daeguense sp. nov., isolated from wastewater of a textile dye works

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A Gram-negative, rod-shaped bacterial strain, K105^T, was isolated from wastewater of a textile dye works in Korea and subjected to a polyphasic taxonomic study. Strain K105^T grew optimally at pH 6.5–7.5 and 30–37 °C. It contained MK-6 as the predominant menaquinone and iso-C_{15:0}, iso-C_{17:1}ω9c, iso-C_{17:0} 3-OH and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) as the major fatty acids. The DNA G+C content was 36.8 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain K105^T belonged to the genus *Chryseobacterium*. Strain K105^T exhibited 16S rRNA gene sequence similarity values of less than 96.9 % to the type strains of recognized *Chryseobacterium* species. Strain K105^T differed from phylogenetically related *Chryseobacterium* species in several phenotypic properties. On the basis of phenotypic and phylogenetic data, strain K105^T (=KCTC 12841^T = JCM 14362^T) is placed in the genus *Chryseobacterium* as the type strain of a novel species, for which the name *Chryseobacterium daeguense* sp. nov. is proposed.

The genus *Chryseobacterium* was proposed by Vandamme *et al.* (1994) and, at the time of writing, the genus comprised 19 recognized species, including the recently described species *Chryseobacterium soldanellicola* and *Chryseobacterium taeanense* (Park *et al.*, 2006), *Chryseobacterium piscium* (de Beer *et al.*, 2006), *Chryseobacterium wanjuense* (Weon *et al.*, 2006), *Chryseobacterium hispanicum* (Gallego *et al.*, 2006), *Chryseobacterium taiwanense* (Tai *et al.*, 2006) and *Chryseobacterium caeni* (Quan *et al.*, 2007). Here we report on the taxonomic characterization of a *Chryseobacterium*-like bacterial strain, K105^T, which was isolated from wastewater of a textile dye works in Daegu, Korea.

Strain K105^T was isolated by using the standard dilution plating technique on trypticase soy agar (TSA; Difco) at 30 °C. The morphological, physiological and biochemical characteristics of strain K105^T were investigated using routine cultivation on TSA at 30 °C. The morphology and flagellation of cells from exponentially growing cultures were examined using light microscopy (Nikon E600) and transmission electron microscopy (Philips CM-20). For the latter purpose, cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after being air-dried. Gliding motility was determined as described by Bowman (2000) using quarter-strength trypticase soy broth (TSB; Difco) solidified with 1 % (w/v) agarose and

R2A agar (Difco). The Gram reaction was determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at various temperatures (4–45 °C) was measured on TSA. Growth in the absence of NaCl and at various NaCl concentrations (0.5 % and 1.0–5.0 %, w/v, at intervals of 1.0 %) was investigated using TSB prepared according to the formula of the Difco medium except that NaCl was excluded. The pH range for growth was determined in nutrient broth (NB; Difco) adjusted, prior to sterilization, to various pH values (pH 4.5–10.5 at intervals of 0.5 pH units) by the addition of HCl or Na₂CO₃. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on plain TSA and on TSA supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically under a nitrogen atmosphere. Catalase and oxidase activities and hydrolysis of casein, gelatin, xanthine, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine and urea were determined as described by Cowan & Steel (1965). DNase activity was examined by using DNase test agar with methyl green (Difco). Hydrolysis of aesculin and nitrate reduction were studied as described previously (Lanyi, 1987). The presence of flexirubin-type pigments was investigated as described by Reichenbach (1992). Susceptibility to antibiotics was tested on TSA plates using antibiotic discs containing the following amounts: polymyxin B, 100 U; streptomycin, 50 µg; penicillin G, 20 U; chloramphenicol, 100 µg; ampicillin, 10 µg; cephalothin, 30 µg; gentamicin, 30 µg; novobiocin, 5 µg; tetracycline, 30 µg; kanamycin, 30 µg; lincomycin, 15 µg; oleandomycin, 15 µg; neomycin, 30 µg; and carbenicillin, 100 µg. Assimilation of and acid production from

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain K105^T is EF076759.

Results from the API 50CH system for strain K105^T are available as supplementary material with the online version of this paper.

various substrates, enzyme activities and other physiological and biochemical properties were tested by using the API 20E, API 20NE, API 50CH and API ZYM systems (bioMérieux). The API 50CH system was inoculated with cell suspensions in API 50 CHB medium and AUX medium for assessing acid production from and assimilation of various substrates, respectively, according to the manufacturer's instructions.

Cell biomass for DNA extraction and for isoprenoid quinone analysis was obtained from cultivation in TSB at 30 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For fatty acid analysis, cell mass of strain K105^T was harvested from TSA plates after incubation for 2 days at 30 °C. The fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G + C content was determined by the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

Morphological, cultural, physiological and biochemical characteristics of strain K105^T are given in the species description (see below), in Table 1 and in Supplementary Table S1 in IJSEM Online. The almost complete 16S rRNA gene sequence of strain K105^T determined in this study comprised 1472 nucleotides, representing approximately

96 % of the *Escherichia coli* 16S rRNA gene sequence. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain K105^T fell among *Chryseobacterium* species (Fig. 1). Strain K105^T exhibited 16S rRNA gene sequence similarity values of 94.4–96.9 % with the type strains of recognized *Chryseobacterium* species and of less than 93.3 % with all other species included in the phylogenetic analysis (Fig. 1).

The predominant isoprenoid quinone detected in strain K105^T was menaquinone-6 (MK-6), at a peak area ratio of approximately 90 %; this is consistent with other *Chryseobacterium* species (Vandamme *et al.*, 1994; Kämpfer *et al.*, 2003; Kim *et al.*, 2005; Tai *et al.*, 2006; Weon *et al.*, 2006). The fatty acid profile of strain K105^T comprised (>0.5 % of total fatty acids) the branched fatty acids iso-C_{15:0} (37.8 %), iso-C_{17:1ω9c} (21.1 %), iso-C_{13:0} (0.7 %), anteiso-C_{15:0} (0.6 %) and iso-C_{17:0} (0.6 %), the hydroxy fatty acids iso-C_{17:0} 3-OH (18.9 %), iso-C_{15:0} 3-OH (4.0 %) and C_{16:0} 3-OH (1.2 %), summed feature 3 (comprising C_{16:1ω7c} and/or iso-C_{15:0} 2-OH; 10.3 %), the straight-chain fatty acid C_{16:0} (0.9 %), the unknown fatty acids ECL 16.582 (1.2 %) and ECL 13.565 (1.0 %) and the unsaturated fatty acid C_{18:1ω5c} (0.8 %). This fatty acid profile was similar to those of other *Chryseobacterium* species, although there were differences in the proportions of some fatty acids, probably because of differences in cultivation conditions and extraction procedures (Vandamme *et al.*, 1994; Kämpfer *et al.*, 2003; Kim *et al.*, 2005; Park *et al.*, 2006; de Beer *et al.*, 2006; Tai *et al.*, 2006; Weon *et al.*, 2006). The DNA G + C content of strain K105^T was 36.8 mol%. Chemotaxonomic data confirm the phylogenetic affiliation of strain K105^T as a member of the genus *Chryseobacterium*. Strain K105^T is distinguishable from phylogenetically related *Chryseobacterium* species by differences in several phenotypic properties, as shown in Table 1. The phylogenetic distinctiveness,

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of *Chryseobacterium daeguense* sp. nov. K105^T, other *Chryseobacterium* species and some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at branching points. *Cytophaga hutchinsonii* ATCC 33406^T (GenBank accession no. M58768) was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

Table 1. Differential phenotypic characteristics of strain K105^T and phylogenetically related *Chryseobacterium* species

Taxa: 1, strain K105^T (*C. daeguense* sp. nov.); 2, *C. daecheongense* (data from Kim *et al.*, 2005); 3, *C. defluvii* (Kämpfer *et al.*, 2003; Kim *et al.*, 2005); 4, *C. taeanense* (Park *et al.*, 2006); 5, *C. taichungense* (Shen *et al.*, 2005; Park *et al.*, 2006; Weon *et al.*, 2006); 6, *C. taiwanense* (Tai *et al.*, 2006); 7, *C. wanjuense* (Weon *et al.*, 2006). +, Positive ; –, negative ; W, weakly positive ; ND, no data available. All species are Gram-negative, non-spore-forming rods. All species are positive for oxidase (not determined for *C. wanjuense*), growth at 37 °C, hydrolysis of aesculin and gelatin, production of flexirubin-type pigments (not determined for *C. taeanense*), alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. All species are negative for motility (not determined for *C. taeanense* or *C. wanjuense*), urea hydrolysis (not determined for *C. taiwanense*), H₂S production (not determined for *C. wanjuense*), acid production from lactose, mannitol, sucrose (not determined for *C. taeanense*) and salicin (not determined for *C. taeanense* or *C. taiwanense*) and α -galactosidase, α -mannosidase and α -fucosidase activities.

Characteristic	1	2	3	4	5	6	7
Growth at:							
5 °C	–	–	–	+	–	+	+
42 °C	–	–	+	–	–	+	–
Growth on MacConkey agar	–	–	–	–	–	ND	W
Indole production	–	–	+	–	W	+	–
Nitrate reduction	–	+	–	–	–	ND	–
Nitrite reduction	–	–	+	–	ND	–	ND
Hydrolysis of:							
Casein	+	+	+	ND	ND	+	+
DNA	+	ND	ND	–	ND	ND	W
Starch	+	+	+	ND	ND	+	+
Acid produced from:							
L-Arabinose	W	–	–	–	–	+	–
D-Cellobiose	–	+	W	ND	–	+	–
D-Fructose	+	+	ND	–	ND	v	–
D-Glucose	+	–	+	ND	W	–	+
Glycerol	–	+	ND	–	ND	–	–
Maltose	+	–	+	–	+	–	–
Raffinose	–	+	–	ND	–	+	–
D-Trehalose	+	+	+	–	+	–	+
D-Xylose	W	+	–	–	+	ND	–
Utilization of:							
L-Arabinose	+	–	–	+	–	ND	–
Glucose	+	+	+	+	–	ND	+
Fructose	+	–	–	+	–	ND	ND
Mannose	+	+	+	+	–	ND	+
Rhamnose	+	–	–	+	–	ND	–
Cellobiose	+	–	–	+	–	ND	ND
Maltose	+	+	+	+	–	ND	+
Sucrose	–	+	–	+	–	ND	–
Trehalose	+	+	+	+	–	ND	ND
Enzyme activities (API ZYM)							
Esterase (C4)	+	–	–	+	+	–	W
Lipase (C14)	–	–	–	–	+	–	–
Cystine arylamidase	–	–	–	–	+	–	W
Trypsin	–	–	–	–	+	–	–
α -Chymotrypsin	–	+	+	–	+	–	W
β -Galactosidase	–	–	–	–	W	–	–
β -Glucuronidase	–	–	–	–	+	–	–
α -Glucosidase	+	–	+	+	+	+	W
β -Glucosidase	–	–	–	+	+	–	–
N-Acetyl- β -glucosaminidase	+	+	+	–	+	–	+
DNA G + C content (mol%)	36.8	36.6	38.8	32.1	ND	36.8	37.8

together with differential phenotypic properties, is sufficient to allocate strain K105^T to a species that is distinct from the recognized *Chryseobacterium* species (Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain K105^T should be classified in the genus *Chryseobacterium* as a member of a novel species, for which the name *Chryseobacterium daeguense* sp. nov. is proposed.

Description of *Chryseobacterium daeguense* sp. nov.

Chryseobacterium daeguense (dae.gu.en'se. N.L. neut. adj. *daeguense* of Daegu, Korea, from where the type strain was isolated).

Cells are Gram-negative, aerobic rods (0.4–0.6 × 0.8–5.0 µm); a few cells are oval or coccoid. No flagellum is detected. Non-motile. Good growth on TSA, nutrient agar and R2A agar; no growth on MacConkey agar. Colonies on TSA are circular, convex, smooth, glistening, light yellow and 2.0–3.0 mm in diameter after 2 days of incubation at 30 °C. The optimal temperature for growth is 30–37 °C. Growth occurs at 10 and 41 °C, but not at 5 or 42 °C. The optimal pH for growth is 6.5–7.5; growth occurs at pH 5.5 and 9.0, but not at pH 5.0 or 9.5. Growth occurs in the presence of 0–2.0 % (w/v) NaCl; optimal growth occurs in the presence of 0–1.0 % (w/v) NaCl. No anaerobic growth is observed on plain TSA or on TSA supplemented with potassium nitrate. Oxidase- and catalase-positive. Tyrosine and Tweens 20, 40, 60 and 80 are hydrolysed, but hypoxanthine and xanthine are not. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. Flexirubin-type pigments are produced. Susceptible to carbenicillin, chloramphenicol, lincomycin, novobiocin, oleandomycin, streptomycin and tetracycline, but not to ampicillin, cephalothin, gentamicin, kanamycin, neomycin, penicillin G or polymyxin B. The predominant menaquinone is MK-6. The major fatty acids (>10 % of total fatty acids) are iso-C_{15:0}, iso-C_{17:1ω9c}, iso-C_{17:0} 3-OH and summed feature 3 (comprising C_{16:1ω7c} and/or iso-C_{15:0} 2-OH). The DNA G + C content is 36.8 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1 and Supplementary Table S1.

The type strain, K105^T (=KCTC 12841^T=JCM 14362^T), was isolated from wastewater collected from a textile dye works in Daegu, Korea.

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