Chryseobacterium shigense sp. nov., a yellow-pigmented, aerobic bacterium isolated from a lactic acid beverage

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A yellow-pigmented bacterium designated strain GUM-Kaji^T was isolated from a lactic acid beverage. The strain had Gram-negative, non-motile, rod-shaped cells. It was strictly aerobic and chemo-organotrophic and grew at 5–30 °C and at pH 5–8. The major components of the non-polar and 3-hydroxy fatty acids were C15:0 iso and 3-OH-C17:0 iso, respectively. Menaquinone MK-6 was detected as the sole quinone. 16S rRNA gene sequence comparisons revealed that strain GUM-Kaji^T is affiliated to the genus *Chryseobacterium*, with *Chryseobacterium joostei* as its phylogenetic neighbour, but there were low levels of similarity (<96%) to any established species of the genus. The G+C content of the genomic DNA was 36.6 mol%. The novel bacterium differed from any known species of *Chryseobacterium* in terms of a number of phenotypic properties. Thus, the name *Chryseobacterium shigense* sp. nov. is proposed for this novel bacterium. The type strain is strain GUM-Kaji^T (=BAMY 1001^T=NCIMB 14047^T=DSM 17126^T).

The genus *Chryseobacterium* was created by Vandamme *et al.* (1994) to accommodate several species formerly classified in the genus *Flavobacterium*, i.e. *Chryseobacterium balustinum*, *C. gleum*, *C. indologenes*, *C. indoltheticum*, *C. meningosepticum* and *C. scopthalmum*. Six species, *Chryseobacterium defluvii* (Kämpfer *et al.*, 2003), *C. joostei* (Hugo *et al.*, 2003), *C. miricola* (Li *et al.*, 2003), *C. daecheongense* (Kim *et al.*, 2005a), *C. formosense* (Young *et al.*, 2005) and *C. taichungense* (Shen *et al.*, 2005), have been added to the genus recently. However, *C. meningosepticum* and *C. miricola* have been transferred to the novel genus *Elizabethkingia* (Kim *et al.*, 2005b) and the genus now consists of 10 species. '*Chryseobacterium proteolyticum*' (Yamaguchi & Yokoe, 2000) is a member of the genus, although the name has not been validly published. From a lactic acid beverage, we isolated a bacterial strain that is phylogenetically and phenotypically similar to *Chryseobacterium* species. In this paper, we report the characteristics

of the novel bacterium and propose to classify it as a novel species of the genus *Chryseobacterium*.

The novel bacterium, designated strain GUM-Kaji^T (NCIMB Japan Culture Collection number BAMY 1001^T), was isolated from a fresh lactic acid beverage (Yumai Co., Ltd, Kouga-gun, Shiga Prefecture, Japan) by the agar-plating method using nutrient agar (CM3; Oxoid). This bacterium has been considered as a part of the normal community in the lactic acid beverage. For testing, precultures were grown aerobically on the same medium. All test media were incubated at 30 °C unless otherwise specified. General cell morphology was studied using phase-contrast and light microscopy (BX50F4; Olympus). Morphological observations under a scanning electron microscope were performed at Hanaichi UltraStructure Research Institute (Okazaki, Japan). The Gram reaction was determined using a Favour G Gram-stain kit (Nissui) according to the manufacturer's instructions. A flexirubin-type pigment was detected by using the method of Reichenbach (1989). Catalase activity, oxidase activity, the oxidation–fermentation reaction, growth on MacConkey agar and the alkaline reaction on Christensen's citrate were determined by using standard methods (Barrow & Feltham, 1993). Additional physiological and biochemical tests were performed using an API 20E kit (bioMérieux) according to the manufacturer's instructions. Fatty acid methyl esters were extracted and prepared by using the

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GUM-Kaji^T is AB193101.

A scanning electron micrograph of cells of strain GUM-Kaji^T is available as a supplementary figure in IJSEM Online.

standard protocol of the Microbial Identification System (MIDI; Microbial ID). Extracts were analysed using a Hewlett Packard model HP6890A gas chromatograph equipped with a flame-ionization detector, according to the manufacturer's instructions. Respiratory quinones were detected by using the HPLC method as described previously (Nishijima *et al.*, 1997). Genomic DNA was extracted and purified using the InstaGene Matrix kit (Bio-Rad). The G + C content (mol%) of the DNA was determined by using the HPLC method, as described previously (Katayama-Fujimura *et al.*, 1984). 16S rRNA gene fragments were PCR-amplified and sequenced using the MicroSeq Full Gene 16S rDNA Bacterial Sequencing kit (Applied Biosystems) and an ABI Model 3100 Genetic Analyzer (Applied Biosystems). The 16S rRNA gene sequence determined was compared with those retrieved from the GenBank/EMBL/DBJ databases. Sequence similarities were calculated and a neighbour-joining evolutionary distance tree (Saitou & Nei, 1987) was constructed using the programs CLUSTAL X (Thompson *et al.*, 1997) and MEGA2 (Kumar *et al.*, 2001). The topology of the tree was evaluated by using the bootstrap resampling method with 1000 replicates (Felsenstein, 1985).

Strain GUM-Kaji^T comprised straight and slightly curved rods measuring 0.6–0.7 µm in width and 1.0–1.5 µm in length (a scanning electron micrograph of cells is available as a supplementary figure in IJSEM Online). The cells were Gram-negative and non-motile. Colonies on nutrient agar were deep-yellow and shiny. A flexirubin-type pigment was detected. No growth occurred on MacConkey agar. The strain was an aerobic chemo-organotrophic bacterium with a strictly respiratory type of metabolism. Growth occurred at 5–30 °C but not at ≥ 37 °C (optimum, 20–30 °C). The pH range for growth was 5–8. Catalase and oxidase activities were present. Casein, gelatin and starch were hydrolysed. Cells were negative for urease activity and nitrate reduction. Acid was produced from D-fructose and D-glucose. Other physiological and biochemical characteristics are shown in the species description.

Cellular fatty acid analysis showed that C15:0 iso was the most abundant component (39.7%). Considerable

proportions of summed feature 4 (2-OH-C15:0 iso and/or C16:1ω7c and/or C16:1ω7t) and C17:1ω9c iso were also found. 3-OH-C17:0 iso (19.6%) was the major component of the 3-hydroxy acids. Analysis of the respiratory quinones revealed that menaquinone MK-6 was the sole quinone.

The 16S rRNA gene sequence of strain GUM-Kaji^T determined (1476 bp) was compared with those retrieved from the databases. The sequence of strain GUM-Kaji^T was most similar to that of *C. joostei* LMG 18212^T (95.7%). The neighbour-joining phylogenetic tree clearly showed that strain GUM-Kaji^T was positioned within the cluster of the genus *Chryseobacterium*, with *C. joostei* as its nearest phylogenetic neighbour (Fig. 1). The G + C content of the genomic DNA of strain GUM-Kaji^T was 36.6 mol%, the value being similar to that of *C. joostei*.

On the basis of the phylogenetic data, it is clear that strain GUM-Kaji^T should be classified as a member of the genus *Chryseobacterium*. This phylogenetic assignment is supported unequivocally by the chemotaxonomic profiles, i.e. by the presence of C15:0 iso and 3-OH-C17:0 iso as the major fatty acids and MK-6 as the sole respiratory quinone. However, strain GUM-Kaji^T differs from all of the previously established species of *Chryseobacterium* in terms of a number of phenotypic characteristics (Table 1). For example, strain GUM-Kaji^T can be differentiated from its closest relative, *C. joostei*, by negative reactions for growth on MacConkey agar and for acid production from maltose and trehalose. Although genomic DNA–DNA reassociation studies were not performed in this study, the low similarities (below 96%) of 16S rRNA gene sequences between our strain and any established *Chryseobacterium* species indicate a distinct position for strain GUM-Kaji^T within the genus, since genomic DNAs of bacterial strains having less than 97.0% sequence similarity will usually not reassociate to more than 60% hybridization (Stackebrandt & Goebel, 1994). In fact, *Chryseobacterium* species sharing 94.9–96.0% 16S rRNA gene sequence similarities showed only 3.0–31.0% DNA–DNA relatedness (Yamaguchi & Yokoe, 2000). DNA binding values as low as 16 and 23% were even found between *C. joostei* and the two other

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain GUM-Kaji^T and previously known species of the genus *Chryseobacterium*. The database 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. *Riemerella anatipestifer* ATCC 11845^T was used as the outgroup to root the tree. Bar, 2% nucleotide substitution rate.

Table 1. Differential characteristics of strain GUM-Kaji^T and previously described *Chryseobacterium* species

Taxa: 1, *C. joostei*; 2, *C. balustinum*; 3, *C. daecheongense*; 4, *C. defluvi*; 5, *C. formosense*; 6, *C. gleum*; 7, *C. indoltheticum*; 8, *C. indologenes*; 9, *C. scophthalmum*; 10, *C. taichungense*; 11, '*C. proteolyticum*'; 12, *Elizabethkingia meningoseptica*; 13, *Elizabethkingia miricola*. Symbols: +, positive; –, negative; W, weakly positive; V, variable reaction; D, delayed reaction; NA, information not available. Data for reference species are based on information from Hugo *et al.* (2003), Li *et al.* (2003), Kim *et al.* (2005a, b), Young *et al.* (2005) and Shen *et al.* (2005).

Characteristic	GUM-Kaji ^T	1	2	3	4	5	6	7	8	9	10	11	12	13
Flexirubin-type pigment	+	+	+	+	+	+	+	+	+	+	+	+	–	–
Growth on/at:														
MacConkey agar	–	+	+	–	–	–	+	+	+	–	–	–	+	+
5 °C	+	+	D	–	–	–	–	+	–	D	NA	NA	–	–
37 °C	–	–	+	+	+	–	+	–	–	–	+	+	+	+
Nitrate reduction	–	–	+	+	–	–	+	–	–	–	–	–	–	–
Starch hydrolysis	+	+	–	+	+	NA	+	–	+	–	NA	+	–	–
Production of:														
Urease	–	V	–	–	–	–	–	–	–	+	–	–	D	+
Indole	+	+	+	–	+	+	+	+	+	–	W	+	D	+
Hydrogen sulphide	–	–	–	–	–	–	–	+	–	–	–	W	–	+
Alkaline reaction on Christensen's citrate	–	V	–	NA	NA	NA	+	+	–	+	NA	NA	D	NA
Acid production from:														
L-Arabinose	–	–	–	–	–	–	+	–	–	–	–	+	–	–
D-Fructose	+	+	+	+	+	NA	+	–	D	–	NA	NA	+	+
D-Glucose	+	+	+	–	+	W	+	+	+	–	W	+	+	+
Glycerol	–	V	–	+	+	NA	+	–	D	–	NA	W	+	–
Lactose	–	–	–	–	–	NA	–	–	–	–	–	–	+	+
Maltose	–	+	–	–	+	–	+	+	+	–	+	+	+	+
D-Mannitol	–	V	–	–	–	–	–	–	–	–	–	W	+	+
Trehalose	–	+	–	+	+	+	+	–	+	+	W	+	+	+
D-Xylose	–	–	–	+	–	W	+	–	–	–	W	NA	–	–
Mean DNA G+C content (mol%)	36.6	36.8	33.1	36.6	33.8	NA	38.0	33.8	38.5	34.2	NA	37.1	37.0	34.6

Chryseobacterium species with which it shared 97.7 and 97.6% 16S rRNA gene sequence similarity, respectively (Hugo *et al.*, 2003). In view of these results, it is logical to conclude that strain GUM-Kaji^T should be classified as a novel species of the genus *Chryseobacterium*, for which we propose the name *Chryseobacterium shigense* sp. nov. Although only one strain of the novel species is available at this time, it is important to create a novel species because of the strain's unique origin and distinct phylogenetic position within the genus.

Description of *Chryseobacterium shigense* sp. nov.

Chryseobacterium shigense (shi.gen'se. N.L. neut. adj. *shigense* pertaining to Shiga Prefecture in Japan, the geographical area of isolation of the type strain).

Cells are straight or slightly curved rods measuring 0.6–0.7 × 1.0–1.5 µm. Gram-negative and non-motile. Strictly aerobic and chemo-organotrophic. Colonies on nutrient agar are deep yellow (flexirubin-type pigment) and shiny. No growth occurs on MacConkey agar. Growth occurs at 5–30 °C (optimum 20–30 °C) but not at 37 °C. The pH range for growth is 5–8. Catalase and oxidase are present.

Indole is produced. Starch, casein and gelatin are hydrolysed. Negative reactions obtained for nitrate reduction, urease activity, hydrogen sulphide production and an alkaline reaction on Christensen's citrate. Acid is produced from D-fructose and D-glucose but not from L-arabinose, D-xylose, glycerol, lactose, maltose, trehalose, D-mannitol or glycerol. The major fatty acids are C15:0 iso and 3-OH-C17:0 iso. Considerable proportions of summed feature 4 (2-OH-C15:0 iso and/or C16:1ω7c and/or C16:1ω7t) and C17:1ω9c iso are also present. Menaquinone MK-6 is the sole respiratory quinone. The G+C content of the genomic DNA of the type strain is 36.6 mol%. The closest phylogenetic relative is *C. joostei*.

The type strain, strain GUM-Kaji^T (=BAMY 1001^T=NCIMB 14047^T=DSM 17126^T), was isolated from a lactic acid beverage in Japan.

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