Chryseobacterium vietnamense sp. nov., isolated from forest soil

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A Gram-reaction-negative bacterial strain, designated GIMN1.005^T, was isolated from a forest soil sample in Vietnam. The isolate was yellow-pigmented, strictly aerobic, and unable to grow below 5 °C or above 37 °C and in the presence of more than 2.0 % NaCl. Cells were non-motile, non-gliding and non-spore-forming. The yellow pigment was of the flexirubin type, non-diffusible and non-fluorescent. Analysis of 16S rRNA gene sequences showed that strain GIMN1.005^T occupied a distinct lineage within the genus *Chryseobacterium*, with sequence similarity values of 98.6, 98.5 and 98.3 % to *Chryseobacterium aquifrigidense* KCTC 12894^T, *Chryseobacterium jejuense* KACC 12501^T and *Chryseobacterium indologenes* GIFU 1347^T, respectively. The level of DNA–DNA relatedness between strain GIMN1.005^T and *C. jejuense* KACC 12501^T was <30 %. The DNA G + C content of strain GIMN1.005^T was 42.1 mol%. The predominant cellular fatty acids were iso-C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3 (C_{16:1}ω7c and/or C_{16:1}ω6c); menaquinone 6 (MK-6) was the sole respiratory quinone. On the basis of phenotypic properties and phylogenetic distinctiveness, strain GIMN1.005^T is considered to represent a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium vietnamense* sp. nov. is proposed. The type strain is GIMN1.005^T (=CCTCC M 209230^T=NRRL B-59550^T).

The genus *Chryseobacterium* (Vandamme *et al.*, 1994; Bernardet *et al.*, 2010) is a member of the family *Flavobacteriaceae*, phylum ‘*Bacteroidetes*’, and, at the time of writing, comprised 47 recognized species (<http://www.bacterio.cict.fr/c/chryseobacterium.html>). *Chryseobacterium* strains are widely distributed in environments such as soil (Weon *et al.*, 2008; Benmalek *et al.*, 2010), rhizosphere (Young *et al.*, 2005; Park *et al.*, 2006), sludge (Kämpfer *et al.*, 2003; Quan *et al.*, 2007; Pires *et al.*, 2010), fish (de Beer *et al.*, 2006; Ilardi *et al.*, 2009), raw dairy products (Hugo *et al.*, 2003; Hantsis-Zacharov *et al.*, 2008), raw chicken (de Beer *et al.*, 2005), water (Yoon *et al.*, 2007), faeces (Kämpfer *et al.*, 2010) and human clinical specimens (Yassin *et al.*, 2010; Bernardet *et al.*, 2006). *Chryseobacterium* strains are Gram-reaction-negative, non-motile, strictly aerobic, yellow-pigmented rods with menaquinone 6 (MK-6) as the major respiratory quinone (Bernardet *et al.*, 2010).

During the course of the characterization of bacterial strains isolated from forest soil at Do Xongpha, Vietnam, a polyphasic approach was used to characterize a Gram-reaction-negative, yellow-pigmented, rod-shaped bacterium. Based on the results of this study, we propose to classify this

strain, designated GIMN1.005^T, as a representative of a novel species of the genus *Chryseobacterium*.

Soil samples were serially diluted in sterile purified water and inoculated on nutrient agar (NA; HuanKai), adjusted to pH 7.0, at 30 °C for 24 h. Strain GIMN1.005^T was isolated on the basis of colony morphology and colour, purified by subculturing and routinely cultured on NA. The culture was suspended in sterile distilled water with 20 % (w/v) glycerol for storage at –80 °C.

The 16S rRNA gene of strain GIMN1.005^T was amplified by PCR with bacterial universal primers 27F and 1527R (Brosius *et al.*, 1981), which were also used for sequencing. The amplified sequenced length was 1390 bp. Analysis of the 16S rRNA gene sequence was performed by using the software package MEGA4.0 (Tamura *et al.*, 2007), after multiple alignment of the data by CLUSTAL X (Thompson *et al.*, 1997; Jukes & Cantor, 1969). Evolutionary distances were used to construct phylogenetic trees via the neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Rzhetsky & Nei, 1992) and maximum-parsimony (Swofford, 1993) methods with bootstrap analysis based on 1000 replications. The neighbour-joining tree is shown in Fig. 1. The topology of the trees generated with the three methods was congruent (data not shown). Strain GIMN1.005^T clearly grouped together with *Chryseobacterium aquifrigidense* KCTC 12894^T. Levels of 16S rRNA gene sequence similarity were

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GIMN1.005^T is HM212415.

Two supplementary figures are available with the online version of this paper.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain GIMN1.005^T and representative members of the genus *Chryseobacterium*. Bootstrap values (percentages of 1000 resampled datasets) are shown at nodes; only values $\geq 70\%$ are shown. Stars indicate that the corresponding nodes were also recovered in the maximum-parsimony and minimum-evolution trees. The sequence of *Bergeyella zoohelcum* ATCC 43767^T was used as an outgroup. Bar, 0.005 nt substitutions per position.

calculated by pairwise alignment via the EzTaxon database (Chun *et al.*, 2007). On this basis, strain GIMN1.005^T was related most closely to *C. aquifrigidense* KCTC 12894^T, *Chryseobacterium jejuense* KACC 12501^T and *Chryseobacterium indologenes* GIFU 1347^T (98.6, 98.5 and 98.3% 16S rRNA gene sequence similarities, respectively). Unfortunately, the type strain of *C. aquifrigidense* could not be obtained from culture collections and was therefore not included as a reference strain in this study. For DNA–DNA hybridization experiments, the genomic DNA of strain GIMN1.005^T was hybridized with that of *C. jejuense* KACC 12501^T. DNA–DNA hybridization experiments were carried out according to De Ley *et al.* (1970). The level of DNA–DNA relatedness between the two strains was $28.6 \pm 0.6\%$ (mean $\pm$ SD of 5 determinations).

Bacterial growth was tested on MacConkey agar and trypticase soy agar (TSA; HuanKai). Unless otherwise stated, all morphological and tolerance tests were performed on NA. The Gram reaction was tested by using a modification of the method described by Gerhardt *et al.* (1994), whereas catalase and cytochrome oxidase tests were performed as described by Smibert & Krieg (1994). Motility was assessed under a Leica DMLB phase-contrast light microscope with Leica Qwin V3 software on cells grown for 1 day in nutrient broth (NB) at 30 °C. For electron microscopy, bacterial cells grown on NA at 30 °C for 24 h and suspended in sterile

purified water were negatively stained with phosphotungstic acid (3%, pH 7.0) for 2 min, air-dried and observed with a Hitachi H7650 electron microscope equipped with the appropriate control system software. The phenotypic tests listed below were performed on strain GIMN1.005^T and on its phylogenetic neighbours *C. jejuense* KACC 12501^T and *C. indologenes* GIFU 1347^T grown under the same conditions. Acid production from sugars was tested as described by Yamaguchi & Yokoe (2000). Nitrate reduction was tested in nitrate broth containing 0.2% KNO₃ (Skerman, 1967). Anaerobic growth was investigated by using incubation in an anaerobic pouch (MGC) for 7 days at 30 °C on NA. The pH range for growth was determined in buffered trypticase soy broth (TSB) adjusted to pH 3–10 (at 1 pH unit intervals). Growth was investigated at 5, 10, 20, 30, 37 and 45 °C in NB. NaCl tolerance was investigated on NA containing 2–10% NaCl (at 1% intervals) at 30 °C. The KOH test was performed according to Bernardet *et al.* (2002) to assess whether the bright yellow pigment produced by strain GIMN1.005^T was of the flexirubin type. Hydrolysis of casein, gelatin, starch, egg yolk, aesculin and tyrosine was investigated according to the methods described by Smibert & Krieg (1994). The production of hydrogen sulphide and indole was assessed by using the triple-sugar-iron method and Ehrlich's and Kovács' reagents, respectively. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase,

β -galactosidase (ONPG) and urease activities were tested as described by Smibert & Krieg (1994). The ability to oxidize 95 substrates was evaluated by using GN2 MicroPlates (Biolog) incubated for 16–24 h at 30 °C according to the manufacturer's instructions.

Cells of strain GIMN1.005^T were non-motile, non-spore-forming rods surrounded by rather thick appendages of regular length (see Fig. S1 available in IJSEM Online). These appendages have not previously been reported for *Chryseobacterium* species and were quite different from the fimbriae reported for *Chryseobacterium flavum* (Zhou *et al.*, 2007). The phenotypic characteristics of strain GIMN1.005^T are given in Table 1 and in the species description below. The following features (taken from Park *et al.*, 2008) distinguish the type strain of *C. aquifrigidense* from strain GIMN1.005^T: inability to grow on MacConkey agar and at 5–10 °C, ability to reduce nitrate and significantly lower DNA G + C content.

For fatty acid analysis, strain GIMN1.005^T, *C. jejuense* KACC 12501^T and *C. indologenes* GIFU 1347^T were grown on TSA for 24 h at 30 °C. Analysis of whole-cell hydrolysates was performed by GLC (Agilent 7890A) according to Kämpfer & Kroppenstedt (1996) by using the classical method of the Sherlock Microbial Identification System version 6.1 (MIDI)

Table 1. Differential characteristics of strain GIMN1.005^T and the type strains of closely related *Chryseobacterium* species

Strains: 1, GIMN1.005^T; 2, *C. indologenes* GIFU 1347^T; 3, *C. jejuense* KACC 12501^T. All data were obtained in this study under the same conditions, except where indicated.

Characteristic	1	2	3
Growth at 37 °C	+	+	–
pH range for growth	6.0–9.0	5.0–8.0	5.0–9.0
Growth in the presence of 3 % NaCl	–	–	+
Production of indole	–	+	–
Urease activity	–	–	+
β -Galactosidase (ONPG)	+	+	–
Acid production from:			
D-Mannitol	+	+	–
L-Fucose	+	–	–
Trehalose	+	–	–
Oxidation of:			
Inositol	+	–	–
L-Alanine	+	+	–
N-Acetyl-D-glucosamine	–	+	–
Propionic acid	+	–	–
Valeric acid	+	–	–
Glycogen	–	+	+
L-Serine	+	+	–
DNA G + C content (mol%)*	42.1	37.6	38.0

*Data for reference strains taken from Weon *et al.* (2008) and Yabuuchi *et al.* (1983).

and the standard MIS library TSBA6. The major fatty acids of strain GIMN1.005^T were iso-C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3 (C_{16:1} ω 7c and/or C_{16:1} ω 6c). The complete fatty acid profile of strain GIMN1.005^T is shown in Table 2 in comparison with those of the type strains of *C. jejuense* and *C. indologenes*. The overall fatty acid composition of the three strains was very similar, with only minor variations in the respective proportions of some components.

Isoprenoid quinones were isolated from lyophilized cells by using the method of Collins *et al.* (1977) and were analysed by HPLC (UltiMate 3000; Dionex) as described by Xie & Yokota (2003). Strain GIMN1.005^T contained menaquinone 6 (MK-6) as the sole menaquinone, which is in line with all other members of the family *Flavobacteriaceae*. The genomic DNA of strain GIMN1.005^T was prepared according to a modification of the procedure described by Wilson (1987). The DNA G + C content of strain GIMN1.005^T, determined by HPLC according to the method of Mesbah *et al.* (1989), was 42.1 mol%, a value significantly higher than those reported for related members of the genus *Chryseobacterium*.

Table 2. Fatty acid contents (%) of strain GIMN1.005^T and the type strains of closely related *Chryseobacterium* species

Strains: 1, GIMN1.005^T; 2, *C. indologenes* GIFU 1347^T; 3, *C. jejuense* KACC 12501^T. tr, Trace (<0.5 %). All data are from this study. Fatty acids amounting to <1 % in all strains tested are not shown.

Fatty acid	1	2	3
iso-C _{13:0}	0.5	tr	0.7
iso-C _{13:0} 3-OH	2.9	tr	tr
iso-C _{15:0}	28.5	30.1	31.3
anteiso-C _{15:0}	0.8	tr	tr
anteiso-C _{15:0} A	1.0	tr	tr
iso-C _{15:0} 3-OH	3.7	2.9	3.1
C _{16:0}	1.8	4.5	4.2
C _{16:0} 3-OH	tr	tr	1.1
iso-C _{16:0} 3-OH	1.0	1.0	tr
iso-C _{17:0}	0.8	3.0	1.2
C _{17:0} 2-OH	0.6	tr	tr
iso-C _{17:0} 3-OH	25.7	18.2	18.1
iso-C _{17:1} ω 9c	9.4	21.7	20.8
C _{18:0}	0.5	tr	2.8
C _{18:1} ω 5c	2.2	0.7	0.5
Summed features*			
1	1.6	tr	tr
3	15.3	12.1	12.0
5	0.8	tr	tr

*Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 1 comprised C_{16:1} ω 7c and/or C_{16:1} ω 6c; summed feature 3 comprised iso-C_{15:0} H and/or C_{13:0} 3-OH; summed feature 5 comprised anteiso-C_{18:0} and/or C_{18:2} ω 6,9c.

On the basis of phenotypic data and phylogenetic inference, it is clear that strain GIMN1.005^T represents a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium vietnamense* sp. nov. is proposed.

Description of *Chryseobacterium vietnamense* sp. nov.

Chryseobacterium vietnamense (vi.et.nam.en'se. N.L. neut. adj. *vietnamense* of or belonging to Vietnam, the country from where the type strain was isolated).

Cells are Gram-reaction-negative, non-motile, non-spore-forming rods approximately 1.0–2.0 µm long and 0.4–0.8 µm in diameter surrounded by thick appendages. Strictly aerobic; oxidase- and catalase-positive. Good growth occurs after 24 h on NA; growth also occurs on MacConkey agar and TSA. Colonies on NA are smooth, bright yellow, circular with entire edges, mucoid, translucent and shiny. Growth occurs at 5–37 °C (optimum, 30 °C), at pH 6–9 (optimum, pH 7) and in the presence of 0–2.0 % NaCl (optimum, 0 %). The non-diffusible and non-fluorescent yellow pigment is of the flexirubin type. Acid is produced from L-fucose, D-glucose, trehalose, D-mannose, D-mannitol and maltose, but not from cellobiose, L-arabinose, D-arabitol, dulcitol, lactose, melibiose, methyl α-D-glucoside, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose or D-xylose. Positive for β-galactosidase activity and for hydrolysis of aesculin, casein, gelatin, starch and tyrosine. Negative for production of indole and hydrogen sulphide and for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and urease activities. The following substrates are oxidized in the GN2 MicroPlate: Tween 80, L-fucose, D-fructose, D-galactose, gentiobiose, α-D-glucose, myo-inositol, α-lactose, lactulose, D-mannose, D-mannitol, maltose, melibiose, D-psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xylitol, methyl pyruvate, monomethyl succinate, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, succinic acid, glucuronamide, L-alaninamide, L-alanine, D-alanine, L-alanyl glycine, L-aspartic acid, L-glutamic acid, glycyl glutamic acid, hydroxy-proline, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-threonine, γ-aminobutyric acid, urocanic acid, inosine, thymidine, uridine, 2,3-butanediol, glycerol, glucose 1-phosphate, glucose 6-phosphate and DL-α-glycerol. The following substrates are not oxidized: α-cyclodextrin, dextrin, glycogen, Tween 40, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, i-erythritol, methyl β-D-glucoside, D-gluconic acid, *p*-hydroxyphenylacetic acid, itaconic acid, DL-lactic acid, sebacic acid, bromosuccinic acid, L-pyrogutamic acid, DL-carnitine, phenylethylamine, putrescine and 2-aminoethanol. The major cellular fatty acids (>15 %) are iso-C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3 (C_{16:1ω7c} and/or C_{16:1ω6c}). MK-6 is the sole respiratory quinone.

The type strain, GIMN1.005^T (=CCTCC M 209230^T =NRRL B-59550^T), was isolated from forest soil in Vietnam. The DNA G+C content of the type strain is 42.1 mol%.

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