Chryseobacterium xinjiangense sp. nov., isolated from alpine permafrost

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Strain TSBY 67^T was isolated during a study on the phylogenetic diversity of culturable bacteria from alpine permafrost in Tianshan Mountains, China. On the basis of 16S rRNA gene sequence analysis, strain TSBY 67^T was closely related to members of the genus *Chryseobacterium* and exhibited 96.8 % 16S rRNA gene sequence similarity to *Chryseobacterium aquaticum* 10-46^T and *Chryseobacterium soldanellicola* PSD 1-4^T. Strain TSBY 67^T grew aerobically, at 4–37 °C, with 0–2 % NaCl and at pH 6–8. Cells were Gram-staining negative, non-motile and non-spore-forming rods. The dominant cellular fatty acids were iso-C_{15:0} (26.9 %), iso-C_{17:0} 3-OH (16.1 %) and iso-C_{17:1ω9c} (15.4 %). The G+C content of the DNA was 33.5 mol%. Strain TSBY 67^T was distinguishable from its closest phylogenetic neighbours by a combination of phenotypic characteristics. Therefore, strain TSBY 67^T represents a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium xinjiangense* sp. nov. is proposed. The type strain is TSBY 67^T (=NRRL B-51308^T=CCTCC AB 207183^T).

The genus *Chryseobacterium* was defined by Vandamme *et al.* (1994) on the basis of rRNA cistron similarity studies and phenotypic characteristics. The genus originally comprised six species: *Chryseobacterium balustinum*, *C. indologenes*, *C. indoltheticum*, *C. meningosepticum*, *C. scopthalmum* and *C. gleum* (type species). At the time of writing, the genus *Chryseobacterium* comprised 47 recognized species (<http://www.bacterio.cict.fr/>). On the basis of phenotypic and genotypic characteristics, *C. meningosepticum* (Vandamme *et al.*, 1994) and *Chryseobacterium miricola* (Li *et al.*, 2003) were transferred to the genus *Elizabethkingia* (Kim *et al.*, 2005) and species of the genera *Sejongia* and *Kaistella* were transferred to the genus *Chryseobacterium* (Kämpfer *et al.*, 2009a, b). Members of the genus *Chryseobacterium* are distributed in a variety of environments, such as soil, fresh water, marine habitats, plants, food products (including raw cow's milk, fish, poultry and lactic acid beverages) and human clinical samples (Shimomura *et al.*, 2005; Park *et al.*, 2006; Vaneechoutte *et al.*, 2007; Hantsis-Zacharov & Halpern, 2007; Hantsis-Zacharov *et al.*, 2008; Ilardi *et al.*, 2009). During a previous study (Bai *et al.*, 2006), strain TSBY 67^T was isolated from a sample of alpine permafrost that was collected at the mouth of an ice-free cirque in the

Tianshan Mountains, China (43° 07' 10.2" N 86° 49' 28.2" E; altitude 3833 m).

For phenotypic tests and fatty acid analysis, the reference strains *Chryseobacterium aquaticum* 10-46^T, *C. soldanellicola* PSD 1-4^T and *C. soli* KACC 12502^T were grown under the same conditions as strain TSBY 67^T. Cell morphology was examined by TEM (JEM 1230; JEOL) using cells grown for 3 days at 20 °C on peptone-yeast extract-glucose (PYG) medium and negatively stained with 1 % phosphotungstic acid (pH 7.0). The Gram reaction was carried out as described by Gerhardt *et al.* (1994). Flexirubin-type pigments were detected with 20 % (w/v) KOH according to the method of Bernardet *et al.* (2002). Growth was investigated on PYG and TSB with 0.5–2 % NaCl (w/v) (at intervals of 0.5 % NaCl) and at 1, 4, 15, 22, 25, 28, 30 and 37 °C for 14 days. Growth at pH 4.0–11 (at intervals of 0.5 pH units) was tested in trypticase soy broth (TSB; Oxoid), with the pH adjusted prior to sterilization by the addition of HCl or NaOH and measured again after sterilization. Growth under anaerobic conditions was determined by incubation in an anaerobic chamber on TSA (Oxoid) supplemented with 0.5 % (w/v) glucose or 0.1 % (w/v) potassium nitrate. Growth was evaluated on TSA, R2A agar, LB agar, nutrient agar and MacConkey agar (Oxoid). Catalase activity was determined by bubble production with 3 % (v/v) H₂O₂ and oxidase activity was determined using 1 % (w/v) N,N,N',N'-tetramethyl *p*-phenylenediamine dihydrochloride (Sigma).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TSBY 67^T is DQ166169.

A supplementary figure is available with the online version of this paper.

Table 1. Characteristics that differentiate strain TSBY 67^T from its closest phylogenetic neighbours in the genus *Chryseobacterium*

Strains: 1, TSBY 67^T; 2, *C. aquaticum* 10-46^T; 3, *C. soldanellicola* PSD1-4^T; 4, *C. soli* KACC 12502^T. All data were taken from this study unless otherwise stated. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4
Growth on MacConkey agar	–	–	–	+
Indole production	+	–	–	–
Urease	–	–	–	+
Enzymic activities				
Trypsin	+	+	–	+
α-Glucosidase	–	–	+	+
N-Acetyl-β-glucosaminidase	+	–	–	+
Hydrolysis of:				
Starch	–	+	+	+
Tween 80	w	w	–	+
DNA G + C content (mol%)*	33.5	38.5	28.8	39.9

*Data for the reference strains were taken from Kim *et al.* (2008), Park *et al.* (2006) and Weon *et al.* (2008).

Biochemical tests were performed using the API 20 E, API 20 NE, API 50 CH and API ZYM systems (bioMérieux) and carbon source utilization was tested using GN2 MicroPlates (Biolog), according to the manufacturers' instructions. Results were recorded after 48 h at 25 °C. Tests for oxidation-fermentation, degradation of DNA, casein, Tweens 20 and 80 and starch were performed using standard methods (Barrow & Feltham, 1993). The morphological, physiological and biochemical characteristics of strain TSBY 67^T are given in Table 1 and the species description.

The 16S rRNA gene sequence of strain TSBY 67^T was amplified using the bacterial universal primers 8f and 1492r, which correspond to positions 8–27 and 1492–1511, respectively, in the 16S rRNA gene sequence of *Escherichia coli*. The almost-complete 16S rRNA gene sequence (1483 bp) was compiled using SeqMan software (DNASTAR) and compared with those of related taxa available in GenBank. Multiple alignments were performed using CLUSTAL_X version 1.81 (Thompson *et al.*, 1997). Evolutionary distances were calculated using the method of Jukes & Cantor (1969) and phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. Tree topologies were evaluated by bootstrap analysis of 1000 replications using MEGA version 4.0 (Tamura *et al.*,

2007). The EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007) was used to calculate sequence similarity. Strain TSBY 67^T showed 96.8% 16S rRNA gene sequence similarity with *C. aquaticum* 10-46^T and *C. soldanellicola* PSD 1-4^T and lower sequence similarities with the type strains of other recognized species of the genus *Chryseobacterium*. In the neighbour-joining phylogenetic tree (Fig. 1), strain TSBY 67^T formed a separate clade within the cluster containing *C. aquaticum* 10-46^T, *C. soldanellicola* PSD 1-4^T and *C. soli* KACC 12502^T. These results demonstrated that the isolate did not belong to any recognized species of the genus *Chryseobacterium* (Stackebrandt & Goebel, 1994; Keswani & Whitman, 2001).

The genomic DNA of strain TSBY 67^T was extracted and degraded enzymically into nucleosides and the DNA G + C content was determined as described by Tamaoka & Komagata (1984) with the following modifications. Nucleosides were separated by HPLC using a Kromasil C₁₈ column (5 µm, 4.6 × 150 mm; Shimadzu) at room temperature. The solvent was 0.6 M NH₄H₂PO₄ (pH 5.6) with 2.5% acetonitrile. The DNA G + C content of strain TSBY 67^T was 33.5 mol%, which was within the range reported for members of the genus *Chryseobacterium*.

The fatty acid methyl esters of strain TSBY 67^T and the three reference strains were obtained from cells grown on TSA at 25 °C for 2 days by saponification, methylation and extraction as described by Kämpfer & Kroppenstedt (1996) and were separated using GC (6890; Agilent). Peaks were automatically integrated and fatty acid identities and percentages were determined using the Sherlock Microbial Identification system (MIDI). The major fatty acids of strain TSBY 67^T were iso-C_{15:0} (26.9%), iso-C_{17:0} 3-OH (16.1%), iso-C_{17:1}ω9c (15.4%), summed feature 3 (consisting of C_{16:1}ω7c and/or C_{16:1}ω6c; 7.2%) and iso-C_{16:0} 3-OH (7.0%). Overall, this composition was consistent with those of the closest phylogenetic neighbours grown under the same conditions (Table 2). However, strain TSBY 67^T differed by having higher proportions of iso-C_{16:0} 3-OH and iso-C_{16:0} and lower proportions of iso-C_{15:0}.

In light of the phylogenetic and phenotypic evidence, strain TSBY 67^T represents a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium xinjiangense* sp. nov. is proposed.

Description of *Chryseobacterium xinjiangense* sp. nov.

Chryseobacterium xinjiangense (xin.jiang.en'se. N.L. neut. adj. *xinjiangense* pertaining to Xinjiang Province, the

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain TSBY 67^T, related members of the genus *Chryseobacterium* and representatives of other related genera in the family *Flavobacteriaceae*. Bootstrap values (>70%) based on 1000 replications are shown at branch nodes. Asterisks indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony method. *Weeksellia virosa* ATCC 43766^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

source of the soil sample from which the type strain was isolated).

Cells are Gram-negative-staining, non-spore-forming, non-motile rods, 0.3–0.5 µm in width and 0.6–1.6 µm in length (see Supplementary Fig. S1, available in IJSEM Online). Strictly aerobic. Oxidase- and catalase-positive. Good growth occurs at 20 °C after 48 h on PYG, TSA, R2A agar, LB agar and nutrient agar, but not on MacConkey agar. Growth within 14 days occurs at 4–37 °C (optimum 20–24 °C), at pH 6–8 (optimum pH 7) and with 0–2 % NaCl (optimum 0.5 % NaCl). Colonies are smooth and shiny, orange–yellow and circular with entire edges and become mucoid and unidentifiable as single entities after prolonged incubation. Flexirubin-type pigments are produced (positive KOH test). The reaction in the oxidation-fermentation test is alkaline. Nitrate and nitrite are not reduced. H₂S and acetoin are not produced. Positive for production of indole and hydrolysis of gelatin, but negative for hydrolysis of aesculin, casein, starch and urea. Arginine dihydrolase, ornithine decarboxylase and lysine

Table 2. Cellular fatty acid compositions of strain TSBY 67^T and its closest phylogenetic neighbours in the genus *Chryseobacterium*

Strains: 1, TSBY 67^T; 2, *C. aquaticum* 10-46^T; 3, *C. soldanellicola* PSD1-4^T; 4, *C. soli* KACC 12502^T. All data were taken from this study. Values are percentages of the total. Fatty acids that amounted to <0.5 % in all strains are not shown. tr, Traces (<0.5 %); ND, not detected/not reported.

Fatty acid	1	2	3	4
iso-C _{13:0}	tr	2.3	1.2	0.7
iso-C _{13:0} 3-OH	0.8	tr	ND	5.4
iso-C _{14:0}	0.9	tr	tr	0.6
iso-C _{15:0}	26.9	36.7	43.2	15.8
C _{15:0} 2-OH	0.5	tr	tr	ND
iso-C _{15:0} 3-OH	3.2	2.4	2.8	1.7
anteiso-C _{15:0}	2.5	2.6	1.8	4.0
iso-C _{15:1} F	0.7	tr	ND	4.6
anteiso-C _{15:1} A	tr	ND	ND	2.5
iso-C _{16:0}	4.3	0.5	tr	3.0
C _{16:0}	3.0	9.9	1.2	9.8
C _{16:0} 3-OH	1.7	5.4	1.4	1.4
iso-C _{16:0} 3-OH	7.0	0.5	tr	2.1
C _{17:0} 2-OH	1.5	0.8	tr	2.1
iso-C _{17:0}	0.6	1.6	0.5	tr
iso-C _{17:0} 3-OH	16.1	14.8	15.8	11.3
iso-C _{17:1} ω9c	15.4	6.8	18.0	8.0
C _{18:1} ω5c	1.3	tr	0.7	tr
C _{18:1} ω9c	ND	ND	ND	4.1
C _{18:0}	2.3	ND	ND	8.2
Summed feature 3*	7.2	11.8	11.2	7.3

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of C_{16:1}ω6c and/or C_{16:1}ω7c.

decarboxylase activities are absent. Acid is not produced from any of the 49 carbon sources in the API 50 CH kit. With API ZYM, alkaline and acid phosphatases, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase and *N*-acetyl-β-glucosaminidase activities are present but lipase (C14), α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase activities are absent. With GN2 MicroPlates, the following compounds are utilized as sole carbon sources: dextrin, glycogen (weakly), Tweens 40 and 80, cellobiose, D-fructose, α-D-glucose (weakly), D-mannose, acetic acid, α-ketobutyric acid, α-ketovaleric acid, propionic acid, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-leucine, L-phenyl-alanine, L-proline, L-serine, L-threonine, inosine, uridine (weakly), thymidine, glucose 1-phosphate (weakly) and D-glucose 6-phosphate (weakly); does not utilize α-cyclodextrin, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, i-erythritol, L-fucose, D-galactose, gentiobiose, *myo*-inositol, lactose, lactulose, maltose, mannitol, melibiose, methyl β-D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xylitol, pyruvic acid methyl ester, succinic acid monomethyl ester, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-, β- and γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketoglutaric acid, DL-lactic acid, malonic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, alaninamide, D-alanine, L-alanine, L-histidine, hydroxy-L-proline, L-ornithine, L-pyroglutamic acid, D-serine, DL-carnitine, γ-aminobutyric acid, urocanic acid, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol or DL-α-glycerol phosphate. The most abundant cellular fatty acids (>7 %) are iso-C_{15:0}, iso-C_{17:0} 3-OH, iso-C_{17:1}ω9c, summed feature 3 (C_{16:1}ω6c and/or C_{16:1}ω7c) and iso-C_{16:0} 3-OH.

The type strain is TSBY 67^T (=NRRL B-51308^T=CCTCC AB 207183^T), isolated from alpine permafrost in Tianshan Mountains, Xinjiang Province, China. The G + C content of the type strain is 33.5 mol%.

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