Brevibacterium album sp. nov., a novel actinobacterium isolated from a saline soil in China

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A novel Gram-positive, rod-shaped actinobacterium, designated strain YIM 90718^T, was isolated from a saline soil in Xinjiang province, north-west China, and subjected to polyphasic taxonomy. The peptidoglycan type was A1 γ and the cell-wall sugars contained galactose. Phospholipids were phosphatidylglycerol and diphosphatidylglycerol. The predominant menaquinone was MK-8(H₂). The major fatty acids were anteiso-C_{15:0}, anteiso-C_{17:0} and iso-C_{15:0}. All of these chemotaxonomic data assigned the new isolate YIM 90718^T consistently to the genus *Brevibacterium*. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain YIM 90718^T formed a distinct phyletic lineage in the genus *Brevibacterium* and showed the highest sequence similarity (96.2%) to *Brevibacterium samyangense* SST-8^T and low similarity (<95.5%) to other species of the genus *Brevibacterium*. On the basis of the polyphasic evidence, a novel species, *Brevibacterium album* sp. nov., is proposed, with the type strain YIM 90718^T (=DSM 18261^T =KCTC 19173^T =CCTCC AB 206112^T).

The genus *Brevibacterium* was first proposed by Breed (1953) for some Gram-positive, non-spore-forming, non-branching rods formerly classified as the genus '*Bacterium*'. The description of the genus was later emended and restricted only to species that correspond to the type species *Brevibacterium linens* on the basis of morphological and chemotaxonomic characteristics (Collins *et al.*, 1980). The genus currently contains 16 species with validly published names isolated from clinical specimens, dairy products, poultry and terrestrial and marine environments. While this paper was in preparation, *Brevibacterium samyangense*, isolated from beach sediment, was reported (Lee, 2006). In this article, strain YIM 90718^T, isolated from a saline soil, is described as a novel member of the genus *Brevibacterium* based on polyphasic taxonomy.

Strain YIM 90718^T was isolated from a saline soil sample collected from Xinjiang, after 2 weeks incubation at 37 °C on modified ISP medium 5. This medium contained (g l⁻¹): L-asparagine, 1.0; glycerol, 10.0; yeast extract, 5.0; K₂HPO₄, 1.0; KNO₃, 5.0; KCl, 150; and agar, 15.0. KCl was

sterilized separately before being added to the medium. The pH of the medium was adjusted to pH 7.5. The strain was maintained on ISP 5 slants (Shirling & Gottlieb, 1966) containing 5% (w/v) NaCl at 4 °C and as glycerol suspensions (20%, v/v) at -20 °C. Biomass for chemical and molecular studies was obtained by cultivation in shaken flasks (about 150 r.p.m.) using ISP medium 5 [5% (w/v) NaCl, pH 7.5] at 37 °C for 1 week.

Cell morphology was determined using cultures grown for 6, 12, 16, 24 and 72 h on modified ISP 5 agar medium supplemented with 5% (w/v) NaCl at 37 °C. Gram staining was carried out by the standard Gram reaction and was confirmed by using the KOH lysis test method (Cerny, 1978). Cell motility was confirmed by the presence of turbidity throughout tubes of semisolid medium (Leifson, 1960). Morphological characteristics of the strain were observed by light microscopy (model BH 2; Olympus) and by transmission electron microscopy with a model H-800 transmission electron microscope (Hitachi). Growth was tested at 4, 10, 20, 28, 37, 45, 55 and 65 °C on ISP medium 5 containing 5% (w/v) NaCl. For NaCl tolerance experiments, modified ISP medium 5 was used as the basal medium. The following NaCl concentrations (w/v) were used: 0, 1, 3, 5, 10, 15, 20, 25 and 30%. The pH range for

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growth was investigated between pH 4.0 and 10.0 at intervals of 1 pH unit using the following buffer systems: pH 4.0–5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0, 0.1 M KH_2PO_4 /0.1 M NaOH; pH 9.0–10.0, 0.1 M NaHCO_3 /0.1 M Na_2CO_3 . Catalase activity was determined by production of bubbles after the addition of a drop of 3 % H_2O_2 . Oxidase activity was observed by oxidation of tetramethyl-*p*-phenylenediamine. Carbon source utilization tests were carried out using GP2 microplates of the Microlog system (Biolog; 95 substrates). Some physiological properties were tested by using the API CORYNE and API ZYM strips (bioMérieux) according to the manufacturer's instructions. The morphological, cultural and physiological properties of strain YIM 90718^T are given in Table 1 and in the species description.

Peptidoglycan was purified and the cell-wall amino acids and peptides in cell-wall hydrolysates were analysed by two-dimensional ascending TLC on cellulose plates using the solvent system of Schleifer & Kandler (1972). The cell-wall sugars were analysed according to the procedures developed by Hasegawa *et al.* (1983). Polar lipids were extracted, examined by two-dimensional TLC and identified using procedures described previously (Minnikin *et al.*, 1984). Menaquinones were isolated according to Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). Cellular fatty acid analysis was performed as described by Sasser (1990) using the Microbial Identification System (MIDI). The peptidoglycan type of YIM 90718^T was A1 γ and galactose was the cell-wall sugar. Phospholipids contained phosphatidylglycerol and diphosphatidylglycerol. The predominant menaquinone was MK-8(H_2). The major fatty acids were anteiso- $\text{C}_{15:0}$, anteiso- $\text{C}_{17:0}$ and iso- $\text{C}_{15:0}$. All of

these features are consistent with the chemotaxonomic description of the genus *Brevibacterium* (Collins *et al.*, 1980). Details of the phospholipids and menaquinones and the cellular fatty acid profile are reported in the species description.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were done as described by Li *et al.* (2007). Multiple alignments with sequences of the most closely related members of *Brevibacterium* and calculations of levels of sequence similarity were carried out using CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic analyses were performed using three tree-making algorithms: the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A phylogenetic tree was constructed using the neighbour-joining method from K_{nuc} values (Kimura, 1980) using MEGA version 2.1 (Kumar *et al.*, 2001). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. Genomic DNA of strain YIM 90718^T for the determination of G+C content was prepared according to the method of Marmur (1961). The G+C content of the DNA was determined by reversed-phase HPLC of nucleosides according to Mesbah *et al.* (1989).

A neighbour-joining phylogenetic tree (Fig. 1) based on 16S rRNA gene sequence comparison clearly showed that strain YIM 90718^T belongs to the genus of *Brevibacterium* and forms a distinct subclade with *B. samyangense*. The 16S rRNA gene sequence similarity between strain YIM 90718^T and its nearest neighbour, *B. samyangense* SST-8^T, was 96.2 %, and low 16S rRNA gene sequence similarity (<95.5 %) was revealed with other species of the genus

Table 1. Differential phenotypic characteristics between strain YIM 90718^T and its nearest phylogenetic neighbour, *B. samyangense* SST-8^T

Data for strain SST-8^T were taken from Lee (2006).

Characteristic	Strain YIM 90718 ^T	<i>B. samyangense</i> SST-8 ^T
Colony colour	White	Bright yellow
Rod-coccus cycle	–	+
Temperature range (°C)	28–45	10–45
pH range	6–8	6.1–10.1
Gelatin hydrolysis	+	–
Utilization of:		
Tween 40	–	+
Sucrose	–	+
α -D-Lactose	–	+
D-Malic acid	+	–
α -Glucosidase	–	+
β -Galactosidase	–	+
α -Chymotrypsin	+	–
Polar lipids*	DPG, PG	PG

*DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol.

Fig. 1. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA gene sequences, showing the position of strain YIM 90718^T and its phylogenetic neighbours. Numbers at branch nodes are bootstrap values (1000 resamplings; only values over 50 % are given). The sequence of *Micrococcus luteus* ATCC 381^T was used as an outgroup. Bar, 1 % sequence divergence.

Brevibacterium. The G+C content of the DNA was 70.0 mol%.

On the basis of the phenotypic, chemotaxonomic and phylogenetic data, strain YIM 90718^T merits recognition within a novel species of the genus *Brevibacterium*, for which we propose the name *Brevibacterium album* sp. nov.

Description of *Brevibacterium album* sp. nov.

Brevibacterium album (al'bum. L. neut. adj. *album* white).

Cells are aerobic, Gram-positive, motile, catalase-positive, oxidase-negative, non-spore-forming rods, 1.8×4.0 – 5.0 μm . Colonies are white on most media tested. Colonies are smooth, circular, opaque and approximately 1–2 mm in diameter after 48 h incubation at 37 °C on ISP medium 5 containing 5 % (w/v) NaCl. Growth occurs in the temperature, pH and salt ranges of 28–45 °C, pH 6–8, 0–10 % NaCl (w/v), 0–20 % KCl (w/v) and 0–30 % $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (w/v). Good growth occurs at 37 °C, pH 7.5, 0–5 % (w/v) NaCl, 0–10 % KCl (w/v) and 0–15 % $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (w/v). The following substrates are utilized as sole carbon sources for growth in Biolog GP2 microplates: β -cyclodextrin, Tween 80, *N*-acetyl-D-glucosamine, L-fucose, D-gluconic acid, *myo*-inositol, lactulose, D-mannitol, methyl β -D-galactoside, methyl α -D-glucoside, palatinose, L-mannose, salicin, trehalose, turanose, D-malic acid, succinic acid, D-alanine, L-alanyl glycine, putrescine, uridine 5'-monophosphate and fructose 6-phosphate. The following substrates are not utilized in the Biolog GP2 system: α -cyclodextrin, dextrin, glycogen, inulin, mannan, Tween 40, *N*-acetyl- β -D-mannosamine, amygdalin, L-arabinose, D-arabitol, arbutin, cellobiose, D-fructose, D-galactose, D-galacturonic acid, gentiobiose, α -D-glucose, α -D-lactose, maltose, maltotriose, D-mannose, melezitose, melibiose, methyl α -D-galactoside, 3-methyl glucose, methyl β -D-glucoside, methyl α -D-mannoside, D-psicose, raffinose, D-ribose, sedoheptulosan, D-sorbitol, stachyose, sucrose, D-tagatose, xylitol, D-xylose, acetic acid, α -, β - and γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α -ketoglutaric acid, α -ketovaleric acid, lactamide, D-lactic acid methyl ester, L-lactic acid, L-malic acid, methyl pyruvate, monomethyl succinate, propionic acid, pyruvic acid, succinamic acid, *N*-acetyl-L-glutamic acid, L-alaninamide, L-alanine, L-asparagine, L-glutamic acid, glycyl L-glutamic acid, L-pyrroglutamic acid, L-serine, 2,3-butanediol, glycerol, adenosine, 2'-deoxyadenosine, inosine, thymidine, uridine, adenosine 5'-monophosphate, thymidine 5'-monophosphate, glucose 1-phosphate, glucose 6-phosphate and DL- α -glycerol phosphate. In the API CORYNE system, tests for pyrazinamidase, pyrrolidonyl arylamidase, alkaline phosphatase, gelatin hydrolysis and acid production from D-ribose are positive. Tests for nitrate reduction, β -glucuronidase, β -galactosidase, α -glucosidase, *N*-acetyl- β -glucosaminidase, β -glucosidase (aesculin hydrolysis), urease and acid production from D-glucose, D-xylose, D-mannitol, maltose, D-lactose, sucrose and glycogen are negative. Using the API ZYM system, acid

and alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin and naphthol-AS-BI-phosphohydrolase tests are positive. Lipase (C14), α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase tests are negative. The peptidoglycan is A1 γ , *meso*-diaminopimelic acid directly cross-linked. Cell-wall sugar contains galactose. The phospholipids are phosphatidylglycerol and diphosphatidylglycerol. The menaquinones are MK-8(H₂), MK-7(H₂), MK-6(H₂), MK-8, MK-6 and MK-9(H₂) (ratio of peak areas, 76:10:5:3:2:1). The fatty acid profile contains anteiso-C_{15:0} (58.71 %), anteiso-C_{17:0} (19.0 %), iso-C_{15:0} (12.29 %) and iso-C_{16:0} (7.24 %). The G+C content of the DNA of the type strain is 70.0 mol%.

The type strain is YIM 90718^T (=DSM 18261^T =KCTC 19173^T =CCTCC AB 206112^T), isolated from a saline soil in the north-west of China.

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