Brevibacterium salitolerans sp. nov., an actinobacterium isolated from salt-lake sediment

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A novel bacterium, designated TRM 415^T, belonging to the genus *Brevibacterium*, was isolated from a sediment sample from a salt lake in Xinjiang province, China. Comparative 16S rRNA gene sequence analysis indicated that strain TRM 415^T was phylogenetically most closely related to *Brevibacterium album* YIM 90718^T (98.4 % sequence similarity) and had low similarity (<95.5 %) to other species of the genus *Brevibacterium*; however, DNA–DNA hybridization studies between strain TRM 415^T and *B. album* YIM 90718^T showed only 41.3 % relatedness. Strain TRM 415^T possessed *meso*-diaminopimelic acid as the diagnostic cell-wall diamino acid, MK-8(H₂) as the major menaquinone and polar lipids including phosphatidylglycerol and diphosphatidylglycerol. The major fatty acids were anteiso-C_{17:0} and anteiso-C_{15:0}. The genomic DNA G + C content was 69 mol%. Based on the evidence from this polyphasic study, strain TRM 415^T represents a novel species of the genus *Brevibacterium*, for which the name *Brevibacterium salitolerans* sp. nov. is proposed. The type strain is TRM 415^T (=JCM 15900^T =CCTCC AB 208328^T =KCTC 19616^T).

The genus *Brevibacterium* was first described by Breed (1953) with *Brevibacterium linens* as the type species; the description was later emended by Collins *et al.* (1980). At the time of writing, the genus *Brevibacterium* contained 21 species with validly published names. In the present study, an actinobacterial isolate, designated TRM 415^T, from a sediment sample collected from Lop Nur salt lake in Xinjiang province, north-west China, was investigated by means of a polyphasic taxonomic approach. The lake environment and precise sampling site location were described previously (Guan *et al.*, 2010).

The sample was isolated on GT2 medium using a 10-fold dilution series method with incubation at 37 °C. The GT2 medium contained (g l⁻¹): glucose (10), yeast extract (1),

tryptone (0.5), CaCO₃ (1), NaCl (50), KCl (30), MgCl₂ (10) and agar (16). The medium was adjusted to pH 7.0–7.5. The organism was grown and maintained on modified ISP 5 medium (Shirling & Gottlieb, 1966) with 2 % (w/v) NaCl, 1 % (w/v) KCl and 0.5 % (w/v) MgCl₂. Biomass for chemical and molecular studies was determined by cultivation in flasks containing the above modified ISP 5 medium and incubation with shaking (~180 r.p.m.) at 37 °C for 1 week.

Cell morphology and motility were examined by optical (Olympus BX40) and transmission electron (JEM-1230) microscopy. Gram staining was carried out using the standard Gram reaction method and was confirmed by using the KOH lysis test (Cerny, 1978). The ability of strain TRM 415^T to grow was tested at 4–55 °C and pH 4–11 as described by Xu *et al.* (2005) and tolerance to concentrations of 0–25 % (w/v) NaCl was tested using ISP 5 as the basal medium. Antimicrobial susceptibility tests were performed using antimicrobial compounds (BBL) according to Mata *et al.* (2002). Carbon source utilization tests were carried out using 95 substrates in GP2 MicroPlates of the Microlog system (Biolog). The physiological properties

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Brevibacterium salitolerans* TRM 415^T is GU117109.

A transmission electron micrograph and a lipid profile of cells of strain TRM 415^T and a phylogenetic tree based on maximum-likelihood analysis of 16S rRNA gene sequences are available as supplementary material with the online version of this paper.

of strain TRM 415^T were tested by using the API Coryne system (bioMérieux) according to the manufacturer's instructions and by using the media and methods of Gordon *et al.* (1974). Detailed morphological, cultural and physiological properties of strain TRM 415^T are given in Table 1 and in the species description.

The type of diamino acid in cell-wall hydrolysates of strain TRM 415^T was determined following methods described previously (Lee, 2006; Stanek & Roberts, 1974). Identification of the cell-wall sugars in whole-cell hydrolysates was performed as described by Hasegawa *et al.* (1983). The absence of mycolic acids was demonstrated with TLC (Minnikin *et al.*, 1980). Polar lipids were extracted, examined by two-dimensional TLC and identified using the procedures of Minnikin *et al.* (1984). Menaquinones were extracted using the method of Collins *et al.* (1977) and analysed by HPLC (Groth *et al.*, 1997). The menaquinones detected included MK-8(H₂) (64.7%), MK-7(H₂) (23.1%), MK-6(H₂) (6.8%), MK-8 (3.4%), MK-7 (1.5%) and MK-6

(0.6%). The major fatty acids were anteiso-C_{17:0} (43.8%) and anteiso-C_{15:0} (30.3%). Cellular fatty acid composition was determined as described by Sasser (1990) using the Microbial Identification System (MIDI). The genomic DNA G + C content of strain TRM 415^T was determined by using the HPLC method (Mesbah *et al.*, 1989). Results of the above experiments are reported in the species description and in Tables 1 and 2. Biomass for chemotaxonomic studies was obtained by growing strain TRM 415^T in flasks containing ISP 5 liquid medium with 2% (w/v) NaCl, 1% (w/v) KCl and 0.5% (w/v) MgCl₂ with shaking at 37 °C for 3 days.

Genomic DNA of strain TRM 415^T was extracted from cells grown on modified ISP 5 medium (as above) for 4 days at 37 °C and used as a template for subsequent PCR amplification. Reaction conditions and amplification protocols were used as described by Li *et al.* (2007). Multiple sequence alignments with closely related members of the genus *Brevibacterium* and sequence similarity calculations were carried out using the CLUSTAL_X program (Thompson

Table 1. Differential characteristics of strains TRM 415^T and *B. album* YIM 90718^T

Strains: 1, TRM 415^T; 2, *B. album* YIM 90718^T

Characteristic	1	2
Colony diameter (mm)	2.0–3.5	1.0–2.5
Colony colour*	WH Y	WH
Motility	–	+
Temperature range (°C)	15–50	22–45
Temperature optimum (°C)	28–40	30–37
NaCl range (% w/v)	0–18	0–13
NaCl optimum (% w/v)	3–8	1–3
pH range	5–9	6–10
Hydrolysis of gelatin	+	–
API Coryne tests:		
Alkaline phosphatase and aesculin hydrolysis	+	–
Susceptibility to:		
Novobiocin	–	+
Chloramphenicol, cefotaxime, cefoxitin and rifampicin	+	–
GP2 tests for utilization of:		
β-Cyclodextrin, N-acetyl-D-glucosamine, myo-inositol, lactulose, D-mannitol, methyl β-D-galactoside, methyl α-D-glucoside, palatinose, salicin, trehalose, turanose, putrescine, uridine 5'-monophosphate and fructose 6-phosphate	–	+
α-Cyclodextrin, dextrin, glycogen, Tween 40, acetic acid, α-, β- and γ-hydroxybutyric acids, α-ketoglutaric acid, α-ketovaleric acid, lactamide, D-lactic acid methyl ester, L-lactic acid, L-malic acid, methyl pyruvate, monomethyl succinate, pyruvic acid, succinamic acid, N-acetyl-L-glutamic acid, L-alaninamide, L-alanine, L-asparagine, L-glutamic acid, glycyl L-glutamic acid, L-serine, 2,3-butanediol, adenosine, 2'-deoxyadenosine, inosine, thymidine and propionic acid	+	–
Presence of MK-7	+	–†
Presence of MK-9(H ₂)	–	–†
Cell-wall sugar(s)‡	Gal, Xyl, Rib	Gal†
DNA G + C content (mol%)	69	70†

*WH, white; WH Y, whitish-yellow.

†Data from Tang *et al.* (2008); data from the current study were obtained using the same experimental conditions.

‡Gal, galactose; Xyl, xylose; Rib, ribose.

Table 2. Cellular fatty acid compositions (%) of strains TRM 415^T and *B. album* YIM 90718^T

Strains: 1, TRM 415^T; 2, *B. album* YIM 90718^T. Fatty acids that account for less than 1.0 % of the total fatty acids in the two strains are not shown. Data are from this study and from Tang *et al.* (2008). NR, Not reported.

Fatty acid	1	2
iso-C _{14:0}	0.4	NR
iso-C _{15:0}	11.0	12.3
anteiso-C _{15:0}	30.3	58.7
iso-C _{16:0}	9.9	7.2
C _{16:0}	0.2	NR
iso-C _{17:0}	4.3	NR
anteiso-C _{17:0}	43.8	19.0

et al., 1997). Phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods using the MEGA 3 program (Kumar *et al.*, 2004) and maximum-likelihood phylogenetic trees were constructed (Felsenstein, 1981) with the PHYLIP 3.6 program. The topology of the resultant phylogenetic trees was evaluated by bootstrap resampling with 1000 replicates (Felsenstein, 1985). The results indicated that strain TRM 415^T clustered within the genus *Brevibacterium* and exhibited the closest phylogenetic affinity (Fig. 1) and highest sequence similarity to

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between the novel isolate TRM 415^T and related taxa. Bootstrap values are percentages based on 1000 replicates; values >50 % are shown. Bar, 0.01 substitutions per nucleotide position.

Brevibacterium album YIM 90718^T (98.4 %) with low similarity (<95.5 %) to other species of the genus *Brevibacterium*. Genomic hybridization experiments between strain TRM 415^T and *B. album* YIM 90718^T were performed by using the method described by He *et al.* (2005). Strain TRM 415^T and *B. album* YIM 90718^T showed 41.3 % DNA–DNA relatedness to each other. Considering 70 % DNA–DNA relatedness as the cut-off point for species delineation (Wayne *et al.*, 1987) and based on the differences in the phenotypic characteristics and the chemotaxonomic and phylogenetic data observed between the two strains, strain TRM 415^T represents a novel species of the genus *Brevibacterium*, for which the name *Brevibacterium salitolerans* sp. nov. is proposed.

Description of *Brevibacterium salitolerans* sp. nov.

Brevibacterium salitolerans (sa.li.to'le.rans. L. n. *sal*, *salis* salt; L. part. adj. *tolerans* tolerating; N.L. part. adj. *salitolerans* salt tolerating, originating from a saline habitat).

Cells are Gram-reaction-positive, aerobic, non-motile rods, approximately 1.3–1.8 × 0.6–1.0 µm. Colonies are 2.0–3.5 mm in diameter, smooth, circular and whitish-yellow when grown, in the dark or in the light, on ISP 5 medium with 2 % (w/v) NaCl, 1 % (w/v) KCl and 0.5 % (w/v) MgCl₂ for 5 days. An apparent rod–coccus growth cycle is not observed after 5 days of growth. Growth occurs at 15–50 °C (optimum 28–40 °C), at pH 5–9 (optimum pH 7.0–7.5) and in concentrations of 0–18 % (w/v) NaCl [optimum 3–8 % (w/v) NaCl]. No growth occurs at 10 or 52 °C or in 20 % (w/v) NaCl. Oxidase-negative and catalase-positive. Nitrate is reduced to nitrite. Gelatin is hydrolysed but Tweens 20, 60 and 80 and starch are not. Negative for milk coagulation and peptonization, cellulose hydrolysis, production of H₂S and methyl red and Voges–Proskauer tests. In the API Coryne system, tests are positive for pyrazinamidase, pyrrolidonyl arylamidase, alkaline phosphatase and β-glucosidase (aesculin hydrolysis) activities, gelatin hydrolysis and acid production from D-ribose; tests are negative for nitrate reduction, β-glucuronidase, β-galactosidase, N-acetyl-β-glucosaminidase, α-glucosidase and urease activities and acid production from D-glucose, D-xylose, D-mannitol, maltose, lactose, sucrose and glycogen. The following substrates are utilized: L-fucose, D-gluconic acid, Tween 80, D-malic acid, succinic acid, D-alanine, L-alanyl glycine, α-cyclodextrin, dextrin, glycogen, Tween 40, acetic acid, α-, β- and γ-hydroxybutyric acids, 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-serine, 2,3-butanediol, adenosine, 2'-deoxyadenosine, inosine and thymidine. The following substrates are not utilized: β-cyclodextrin, N-acetyl-D-glucosamine, myo-inositol, lactulose, D-mannitol, methyl

β -D-galactoside, methyl α -D-glucoside, palatinose, L-rhamnose, salicin, trehalose, turanose, putrescine, uridine 5'-monophosphate, fructose 6-phosphate, inulin, mannan, N-acetyl- β -D-mannosamine, amygdalin, L-arabinose, D-arabitol, arbutin, cellobiose, D-fructose, D-galactose, D-galacturonic acid, gentiobiose, α -D-glucose, lactose, maltose, 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, *p*-hydroxyphenylacetic acid, L-pyroglutamic acid, glycerol, uridine, adenosine 5'-monophosphate, thymidine 5'-monophosphate, glucose 1-phosphate, glucose 6-phosphate and DL- α -glycerol phosphate. Susceptible to (μ g unless stated otherwise) streptomycin (10), amoxicillin (25), chloramphenicol (30), cefoxitin (30), kanamycin (30), nalidixic acid (30), nitrofurantoin (300), ampicillin (10), sulfamide (250), cefotaxime (30), erythromycin (15), polymyxin B (300 UI), rifampicin (30) and carbenicillin (100), but resistant to tobramycin (10) and trimethoprim-sulfamethoxazole (1.25–23.75). Whole-cell hydrolysates contain *meso*-diaminopimelic acid. Mycolic acids are absent. Cell-wall sugars contain galactose, xylose and ribose and the phospholipids include phosphatidylglycerol and diphosphatidylglycerol. The menaquinones are MK-8(H₂), MK-7(H₂), MK-6(H₂), MK-8, MK-7 and MK-6. The major fatty acids are anteiso-C_{17:0} and anteiso-C_{15:0}. The genomic DNA G + C content of the type strain is 69 mol%.

The type strain, TRM 415^T (=JCM 15900^T =CCTCC AB 208328^T =KCTC 19616^T), was isolated from a soil sediment sample from a hypersaline lake habitat in Xinjiang province, north-west China.

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