Georgenia halophila sp. nov., a halophilic actinobacterium isolated from a salt lake

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A Gram-stain-positive actinobacterium, designated strain YIM 93316^T, was isolated from a salt lake in Xinjiang Province, north-west China, and was subjected to a polyphasic taxonomic study. The isolate grew at 10–45 °C, at pH 6–9 and in the presence of 1–15 % (w/v) NaCl, but no growth was observed in the absence of NaCl. The cell-wall diamino acid contained alanine, glutamine and lysine with peptidoglycan type A4 α . Polar lipids contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, an unknown glycolipid and an unknown phospholipid. The predominant menaquinone was MK-8(H₄). The major fatty acids were anteiso-C_{15:0} and anteiso-C_{15:1}. The DNA G + C content of strain YIM 93316^T was 70.1 mol%. Chemotaxonomic properties supported the affiliation of strain YIM 93316^T to the genus *Georgenia*, and this was supported by phylogenetic analysis based on 16S rRNA gene sequences. Levels of 16S rRNA gene sequence similarity between strain YIM 93316^T and *Georgenia thermotolerans* TT02-04^T, *Georgenia ruanii* YIM 004^T and *Georgenia muralis* 1A-C^T were 96.6, 96.5 and 96.3 %, respectively. Data from fatty acid, physiological and biochemical tests allowed the clear phenotypic differentiation of strain YIM 93316^T from recognized members of the genus *Georgenia*. On the basis of evidence from the present polyphasic study, strain YIM 93316^T is considered to represent a novel species of the genus *Georgenia*, for which the name *Georgenia halophila* sp. nov. is proposed. The type strain is YIM 93316^T (=DSM 21365^T =CCTCC AB 208144^T).

The genus *Georgenia* was proposed by Altenburger *et al.* (2002) with the description of *Georgenia muralis* as the type species, and the description was later emended by Li *et al.* (2007). At the time of writing, the genus comprises three recognized species, namely *G. muralis* (Altenburger *et al.*, 2002), *Georgenia ruanii* (Li *et al.*, 2007) and *Georgenia thermotolerans* (Hamada *et al.*, 2009). Members of the genus *Georgenia* are Gram-positive, aerobic or facultatively anaerobic, non-endospore-forming, oxidase- and catalase-positive, motile or non-motile actinobacteria, with pepti-

doglycan type A4 α (lysine as the diagnostic diamino acid), MK-8(H₄) as the predominant menaquinone, anteiso-C_{15:0} as the major fatty acid and DNA G + C content of 70–73 mol%.

Strain YIM 93316^T was isolated from a soil sample collected from Qijiaoqing Lake in Xinjiang Province, north-west China (GPS coordinates for the sampling site are 43° 23' 01" N 91° 36' 11" E), after 3 weeks of incubation at 37 °C on glucose-tryptone-yeast (GTY) medium [per litre distilled water: 1 g glucose, 0.5 g tryptone, 2 g yeast extract, 1 g CaCl₂, 100 g NaCl and 15 g agar; final pH 7.5]. Strain YIM 93316^T was maintained on GTY agar slants containing 10 % NaCl (w/v) 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.) with GTY liquid medium [10 % (w/v) NaCl, pH 7.5] at 37 °C for 1 week.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 93316^T is FJ717681.

Chromatograms of O-phthalaldehyde- and 1-phenyl-3-methyl-5-pyrazolone-derivatization samples of cell walls of strain YIM 93316^T and a figure showing the polar lipid composition of strain YIM 93316^T are available as supplementary material with the online version of this paper.

Cell morphology was determined after 6, 12, 24, 48 and 72 h incubation on GTY agar medium supplemented with 10 % (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 based on the presence of turbidity throughout the tube including semisolid medium (Leifson, 1960). Morphological characteristics of the novel strain were observed by light microscopy (model BH 2; Olympus) and by transmission electron microscopy (model H-800; Hitachi). Growth was tested at 4–55 °C on GTY medium containing 10 % (w/v) NaCl, at intervals of 5 °C. For NaCl tolerance experiments, GTY medium was used as the basal medium with salt concentrations from 0 to 20 % (w/v), at intervals of 1 %. The pH range for growth was investigated between pH 4.0 and 10.0 at intervals of 1 pH unit. The buffer system was as follows: pH 4.0–5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0, 0.1 M KH₂PO₄/0.1 M NaOH; pH 9.0–10.0, 0.1 M NaHCO₃/0.1 M Na₂CO₃. Catalase activity was determined by production of bubbles after the addition of a drop of 3 % H₂O₂. Oxidase activity was observed by oxidation of tetramethyl-*p*-phenylenediamine. Hydrolysis of aesculin, casein, chitin, cellulose, dextrin, gelatin, starch and Tweens 20, 40, 60 and 80 was determined as described by Cowan & Steel (1965). Physiological and biochemical tests were performed by using the API ZYM, API Coryne, API 20E and API 50CHB systems (bioMérieux) according to the manufacturer's instructions. Inocula were prepared by using pre-warmed sterile saline medium (10 % NaCl), within the density range specified by the manufacturer. API 50CHB and API ZYM tests were performed in duplicate. Anaerobic growth experiments were performed in GTY medium supplemented with 0.15 mM nitrate and 0.035 mM nitrite by using a GasPak Anaerobic System (BBL) according to the manufacturer's instructions. The detailed physiological and biochemical properties of strain YIM 93316^T are given in Table 1 and in the species description.

The peptidoglycan of cells of strain YIM 93316^T was isolated after disruption of the cells by shaking with glass beads and subsequent trypsin digestion, according to the method of Schleifer (1985). The cell wall was hydrolysed for amino acid and sugar analyses as described by Schleifer & Kandler (1972). Amino acids in cell-wall hydrolysates were analysed by precolumn derivatization with *O*-phthalaldehyde by HPLC (Tang *et al.*, 2009a, b). *G. ruanii* YIM 004^T was used as the reference strain for analysis of cell-wall amino acids. Whole-cell sugars were detected by precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone by HPLC (Tang *et al.*, 2009b). Polar lipids were extracted, examined by two-dimensional TLC and identified by using published procedures (Minnikin *et al.*, 1984). Menaquinones were isolated according to the method of Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). For analysis of fatty acids, strain YIM 93316^T was cultured on trypticase soy agar (TSA; Difco) containing 10 % NaCl at 37 °C for 72 h.

Preparation and analysis of fatty acid methyl esters were performed as described by Sasser (1990) by using the Microbial Identification System (MIDI).

The cell-wall diamino acid of strain YIM 93316^T contained glutamine, alanine and lysine (Supplementary Fig. S1, available in IJSEM Online), which is similar to results for *G. ruanii* YIM 004^T and is characteristic of the genus *Georgenia* (peptidoglycan type A4 α , murein type A11.54; http://www.dsmz.de/microorganisms/main.php?content_id=35). Major whole-cell-wall sugars were rhamnose (38.2 %), glucosamine (31.7 %) and galactose (10.3 %); minor amounts of mannose (7.1 %), glucose (6.3 %) and xylose (6.4 %) were detected (Supplementary Fig. S2). The polar lipids contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, an unknown glycolipid and an unknown phospholipid (Supplementary Fig. S3). The predominant menaquinone was MK-8(H₄). Strain YIM 93316^T had a cellular fatty acid profile that contained large amounts of branched fatty acids; the major components (>10 % of the total) were anteiso-C_{15:0} (69.8 %) and anteiso-C_{15:1} (14.2 %). Minor fatty acids detected were iso-C_{14:0} (1.6 %), C_{14:0} (1.9 %), iso-C_{15:0} (6.1 %), C_{16:0} (1.6 %), anteiso-C_{17:0} (3.4 %) and C_{18:0} (1.4 %).

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were performed as described by Li *et al.* (2007). Multiple alignments with sequences of the type strains of *Georgenia* species and calculations of levels of 16S rRNA gene sequence similarity were carried out by using the EzTaxon server 2.0 (Chun *et al.*, 2007). Phylogenetic analyses were performed by using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A phylogenetic tree was constructed with the neighbour-joining method of Saitou & Nei (1987) from *K*_{nuc} values (Kimura, 1980) by using MEGA version 4.0 (Tamura *et al.*, 2007). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. Genomic DNA of strain YIM 93316^T for 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).

Searches with the BLAST program showed that strain YIM 93316^T had highest 16S rRNA gene sequence similarity to members of the genus *Georgenia*. A phylogenetic tree was constructed with sequences of representative strains of the genus *Georgenia*, and *Brevibacterium linens* DSM 20425^T was used as the outgroup. In the phylogenetic tree based on the neighbour-joining algorithm, strain YIM 93316^T formed a monophyletic clade within the evolutionary radiation occupied by the genus *Georgenia* (Fig. 1). Similar topologies were found in the maximum-likelihood and maximum-parsimony trees. Levels of 16S rRNA gene sequence similarity between strain YIM 93316^T and *G. thermotolerans* TT02-04^T, *G. ruanii* YIM 004^T and *G.*

Table 1. Differential characteristics between strain YIM 93316^T and members of the genus *Georgenia*

Strains: 1, YIM 93316^T; 2, *G. thermotolerans* TT02-04^T (data from Hamada *et al.*, 2009); 3, *G. ruanii* YIM 004^T (Li *et al.*, 2007; Hamada *et al.*, 2009); 4, *G. muralis* 1A-C^T (Altenburger *et al.*, 2002; Hamada *et al.*, 2009). +, Positive; –, negative; w, weakly positive, ND, no data available.

Characteristic	1	2	3	4
Cell morphology*	SR	SR	SR	R–C
Colony colour†	Y	WHY	WHY–ST	Y
Motility	–	+	+	–
Growth at 45 °C	+	+	–	–
Growth in:				
0 % NaCl	–	+	+	+
7 % NaCl	+	+	–	+
15 % NaCl	+	–	–	–
API ZYM results				
Alkaline phosphatase	+	–	–	+
Naphthol-AS-BI-phosphohydrolase	+	+	–	–
β-Galactosidase	+	–	–	+
N-Acetyl-β-glucosaminidase	–	–	+	–
API Coryne results				
Nitrate reduction	–	+	–	+
Pyrazinamidase	+	+	+	–
Pyrrolidonyl arylamidase	–	+	–	w
Aesculin hydrolysis	+	–	–	+
Acid production from (API 50CH):				
L-Arabinose	+	–	–	+
D-Ribose	+	+	+	–
L-Xylose	–	–	–	+
D-Mannitol	–	–	–	+
Methyl α-D-glucopyranoside	+	+	–	–
Amygdalin	–	+	–	+
Arbutin	–	+	–	+
Salicin	+	+	–	+
Melibiose	–	–	–	+
Trehalose	–	–	+	+
Raffinose	–	–	–	+
Starch	–	–	–	+
Glycogen	–	–	–	+
Cell-wall sugar(s)‡	Rha, GlcN, Gal	Gal	Gal, Rha	ND
Polar lipids§	DPG, PG, 1PL, PI, PIM, GL	DPG, PG, PIM	DPG, PG, PI, 1PL	DPG, PG, PIM, 2PLs, GL
Major fatty acid(s) (>10 %)	ai-C _{15:0} , ai-C _{15:1}	ai-C _{15:0}	ai-C _{15:0}	ai-C _{15:0}
DNA G + C content (mol%)	70.1	73	72.9	70.0

*R–C, Rod–coccus cycle; SR, short rods.

†ST, Straw-coloured; WHY, whitish yellow; Y, yellow.

‡Gal, Galactose; GlcN, glucosamine; Rha, rhamnose.

§DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PL, unknown phospholipid; GL, unknown glycolipid.

muralis 1A-C^T were 96.6, 96.5 and 96.3 %, respectively. The G + C content of the DNA of strain YIM 93316^T was 70.1 mol%.

The chemotaxonomic properties of strain YIM 93316^T [peptidoglycan type A4α; anteiso-C_{15:0} as the major fatty acid; diphosphatidylglycerol and phosphatidylglycerol as major phospholipids; MK-8(H₄) as the predominant

menaquinone] indicated an affiliation to the genus *Georgenia* (Altenburger *et al.*, 2002; Li *et al.*, 2007; Hamada *et al.*, 2009). 16S rRNA gene sequence analysis also indicated that strain YIM 93316^T had closest phylogenetic affiliation to the genus *Georgenia*, which was confirmed by a high bootstrap resampling value. Therefore, both chemotaxonomic and phylogenetic results clearly indicated that strain YIM 93316^T belongs to the genus

Fig. 1. Neighbour-joining phylogenetic dendrogram obtained by distance-matrix analysis of 16S rRNA gene sequences, showing the position of strain YIM 93316^T and its phylogenetic neighbours. Asterisks indicate branches of the tree that were also found by using the maximum-likelihood and maximum-parsimony tree-making algorithms. Numbers at nodes are bootstrap values (percentages based on 1000 resamplings, only values >50% are given). The sequence of *Brevibacterium linens* DSM 20425^T was used as the outgroup. Bar, 1% sequence divergence.

Georgenia. However, YIM 93316^T could be differentiated from members of the genus *Georgenia* based on several morphological, physiological and chemotaxonomic properties (Table 1). In particular, strain YIM 93316^T was a halophilic actinobacterium; growth was observed at up to 15% NaCl, but no growth occurred in the absence of NaCl. By contrast, recognized members of the genus *Georgenia* are non-halophilic actinobacteria that are able to grow without NaCl and do not grow in the presence of 15% NaCl. Furthermore, strain YIM 93316^T exhibited differences from recognized members of the genus *Georgenia* in its fatty acid composition. For instance, strain YIM 93316^T contained anteiso-C_{15:0} and anteiso-C_{15:1} as major fatty acids (>10%), whereas recognized *Georgenia* species do not have anteiso-C_{15:1} as a major component.

Therefore, on the basis of its phenotypic and genotypic distinctiveness, strain YIM 93316^T is considered to represent a novel species of the genus *Georgenia*, for which we propose the name *Georgenia halophila* sp. nov.

Description of *Georgenia halophila* sp. nov.

Georgenia halophila (ha.lo.phi'la. Gr. n. *hals*, *halos* salt; Gr. adj. *philos* loving; N.L. fem. adj. *halophila* salt-loving, referring to the ability of the type strain to grow at high NaCl concentrations).

Cells are Gram-stain-positive, facultatively anaerobic, non-motile, non-endospore-forming, short rods (0.2–0.5 µm in

diameter). Colonies are yellow, circular, opaque, slightly convex, smooth and approximately 1.0 mm in diameter after 24 h at 37 °C. No water-soluble exopigment is formed. Grows at 10–45 °C, at pH 6.0–9.0 and in the presence of 1–15% (w/v) NaCl; no growth is observed at 4 or 50 °C, above pH 10 or in the presence of 16% NaCl; optimal growth occurs in the presence of 5–10% NaCl, at pH 7.0–8.0 and at 37 °C. Oxidase- and catalase-positive. Voges-Proskauer and methyl red tests are negative. Positive for hydrolysis of starch, aesculin and Tween 80, but negative for hydrolysis of gelatin, chitin, cellulose, dextrin, urea, casein and Tweens 20, 40 and 60. Indole and H₂S are not produced. Nitrate reduction is positive. In the API 50CHB system, acid is produced from glycerol, D- and L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-mannose, L-rhamnose, methyl α-D-mannopyranoside, aesculin, salicin, sucrose, D-lyxose, L-fucose and potassium 5-ketogluconate, but not erythritol, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-fructose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, cellobiose, maltose, lactose, melibiose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-tagatose, D-fucose, DL-arabitol, potassium gluconate or potassium 2-ketogluconate. In the API Coryne system, positive for alkaline phosphatase, N-acetyl-β-glucosaminidase, pyrazinamidase and aesculin hydrolysis, but negative for nitrate reduction, α-glucosidase, β-glucosidase, pyrrolidonyl arylamidase, β-glucuronidase, β-galactosidase, urease and gelatin hydrolysis. In the API ZYM system, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucosidase and α-mannosidase, but negative for valine arylamidase, cystine arylamidase, trypsin, α-glucosidase, lipase (C14), α-chymotrypsin, acid phosphatase, β-glucuronidase, N-acetyl-β-glucosaminidase and α-fucosidase. The peptidoglycan type is A4α. The major whole-cell-wall sugars are rhamnose, glucosamine and galactose. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, an unknown glycolipid and an unknown phospholipid. The predominant menaquinone is MK-8(H₄). The major cellular fatty acids are anteiso-C_{15:0} and anteiso-C_{15:1}; minor straight-chain fatty acids are detected. The G+C content of the DNA of the type strain is 70.1 mol%.

The type strain, YIM 93316^T (=DSM 21365^T =CCTCC AB 208144^T), was isolated from a salt lake sample collected from Qijiaoing Lake in Xinjiang Province, north-west China.

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