Georgenia satyanarayanai sp. nov., an alkaliphilic and thermotolerant amylase-producing actinobacterium isolated from a soda lake

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A Gram-stain-positive, oxidase-negative, starch-hydrolysing, actinobacterium (strain JC82^T) was isolated from a soda lake in Lonar, India. Based on 16S rRNA gene sequence similarity studies, strain JC82^T belonged to the genus *Georgenia* and was most closely related to *Georgenia muralis* 1A-C^T (96.8%) and other members of the genus *Georgenia* (<96.5%). The DNA G+C content of strain JC82^T was 73.4 mol%. The cell-wall amino acids were alanine, glutamic acid and lysine with peptidoglycan type A4 α . Polar lipids included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, an unidentified lipid (L1) and an unidentified glycolipid (GL3). The predominant isoprenoid quinone was menaquinone MK-8(H₄). Anteiso-C_{15:0} was the predominant fatty acid and significant proportions of iso-C_{14:0}, C_{14:0}, C_{16:0}, iso-C_{15:0} and iso-C_{16:0} were also detected. Strain JC82^T produced thermostable alkaline amylase. The results of physiological and biochemical tests allowed a clear phenotypic differentiation of strain JC82^T from all other members of the genus *Georgenia*. Based on these data, strain JC82^T represents a novel species of the genus *Georgenia*, for which the name *Georgenia satyanarayanai* sp. nov. is proposed. The type strain is JC82^T (=KCTC 19802^T =NBRC 107612^T).

The alkaline Lonar Lake (19° 58' 36" N, 76° 30' 30" E) in India is a unique basaltic rock meteorite impact crater, ranking third in the world in terms of alkalinity. The lake is rich in bacterial diversity (Joshi *et al.*, 2008; Wani *et al.*, 2006), although at the time of writing, only two bacterial species isolated from the lake had validly published names (*Indibacter alkaliphilus* and *Nitritalea halalkaliphila*; Anil Kumar *et al.*, 2010a, b). In this report, we describe a novel actinobacterium, strain JC82^T belonging to the genus *Georgenia* which was isolated from a sediment sample from Lonar Lake. The genus *Georgenia* was first proposed by Altenburger *et al.* (2002) with *Georgenia muralis* as the type species. At the time of writing, the genus *Georgenia* comprised six recognized species, including the recently described *Georgenia daeguensis* (Woo *et al.*, 2012). The members of this genus are Gram-stain-positive, aerobic or facultative anaerobic, non-sporulating, oxidase- and catalase-positive, motile or non-motile bacteria

with MK-8(H₄) as the predominant menaquinone, anteiso-C_{15:0} as the major fatty acid, lysine as the diagnostic cell wall amino acid and a polar lipid profile with diphosphatidylglycerol and phosphatidylglycerol as major components (Tang *et al.*, 2010).

Strain JC82^T was isolated from a sediment sample collected from Lonar Lake in Maharashtra, India, using nutrient agar (Hi-Media) under aerobic conditions. Pure cultures were lyophilized and preserved at 4 °C. Morphological properties such as cell shape, cell size and motility were observed by phase-contrast light microscopy (BH-2; Olympus). The pH and temperature ranges for growth were examined in nutrient broth. Salt tolerance tests were carried out in nutrient broth supplemented with varying concentrations of NaCl. Growth was measured turbidometrically at 540 nm in a colorimeter (Systronics). Various biochemical tests such as hydrolysis of starch and gelatin, and chitinase, oxidase and catalase activities were carried out in media described by Cappuccino & Sherman (1998). Other biochemical tests were performed using the GP2 MicroPlate (Biolog) according to the manufacturer's instructions. *Georgenia muralis* 1A-C^T (=JCM 12241^T) and *Georgenia soli* CC-NMPT-T3^T (=DSM 21838^T) (obtained from Dr C.-C.

Abbreviations: ME, minimum-evolution; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JC82^T is FN995635.

Four supplementary figures and a supplementary table are available with the online version of this paper.

Young, National Chung Hsing University, Taiwan) were used for comparative taxonomic analysis throughout this study as recommended for minimum standards for description of new species (Tindall *et al.*, 2010).

Fatty acids, quinones and polar lipids of the strains were analysed from cells grown in nutrient broth. Cells were harvested by centrifugation (10 000 g for 15 min at 4 °C) after reaching a cell density of 70 % of the maximum optical density (100 % = 0.8 OD₅₄₀), and the lyophilized pellet was used for analysis. Cellular fatty acids were methylated, separated and identified according to the instructions for the Microbial Identification System [MIDI version 6.0; Microbial ID (Sasser, 1990)] which was outsourced through Royal Research Laboratories, Secunderabad, India.

Polar lipids were extracted from 1 g freeze-dried cells with methanol/chloroform/saline (2 : 1 : 0.8, by vol.) as described by Kates (1986). Lipids were separated by two-dimensional thin layer chromatography (TLC) using silica gel TLC plates (Kieselgel 60 F₂₅₄; Merck) with chloroform/methanol/water (75 : 32 : 4, by vol.) in the first dimension and chloroform/methanol/acetic acid/water (86 : 16 : 15 : 4, by vol.) in the second dimension (modified after Tindall, 1990a, b; Oren *et al.*, 1996). Total polar lipid profiles were detected by spraying with 5 % (v/v) ethanolic molybdophosphoric acid and further characterized by spraying with ninhydrin (for amino groups), molybdenum blue (for phosphates), Dragendorff's reagent (for quaternary nitrogen) or α -naphthol (for sugars) (Kates, 1972; Oren *et al.*, 1996). Quinones were extracted with a chloroform/methanol (2 : 1, v/v) mixture, purified by TLC and analysed by HPLC (Hiraishi & Hoshino, 1984; Hiraishi *et al.*, 1984).

The peptidoglycan of cells of strain JC82^T was isolated following 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 analyses as described by Schleifer & Kandler (1972). Amino acids in cell-wall hydrolysates were analysed by HPLC (Tang *et al.*, 2009a, b; McKerrow *et al.*, 2000).

For analysis of amylase activity bacterial strains were grown on starch (2 % w/v) as sole source of carbon in a mineral medium which contained (g l⁻¹): NH₄Cl (0.6); KH₂PO₄ (0.5); NaCl (0.4); MgSO₄ · 7H₂O (0.24), CaCl₂ (0.05), and yeast extract (0.3). Cells were harvested by centrifugation (10 000 r.p.m. for 20 min at 4 °C) after 48 h of growth, and the supernatant was used as a source for amylase. Ammonium sulphate was added to the supernatant to achieve 90 % saturation, keeping pH at 7.0. Precipitated proteins were separated by centrifugation (30 000 g for 20 min), resuspended in phosphate buffer (pH 7) and dialysed against three changes of 3 l of the same buffer for 3 h each. Amylase activity was determined by measuring the formation of reducing sugars (analysed according to Nelson, 1944) from starch (1 %) in a final volume of 0.5 ml of an appropriate buffer (K₂HPO₄-KH₂PO₄ buffer for pH 5–8; NaHCO₃-NaOH buffer for pH 9–11; and NaCO₃-NaOH buffer for pH 12). A unit (U) of amylase is defined

as the amount of enzyme that released 1 μ M min⁻¹ reducing sugars.

Genomic DNA was extracted and purified according to the method of Marmur (1961) and the G+C content (mol%) of the DNA was determined by HPLC (Mesbah *et al.*, 1989). Cell material for 16S rRNA gene sequencing was taken from a single colony. DNA was extracted and purified by using a Qiagen genomic DNA extraction kit. Recombinant *Taq* polymerase (Genei) was used for PCR. The complete length of the 16S rRNA gene sequence was obtained by sequencing with primers, F'-27 (5'-GTTTGA-TCCTGGCTCAG-3'), and R'-1489 (5'-TACCTTGTTAC-GACTTCA-3') [corresponding to positions 11–27 and 1489–1506 for F'-27 and R'-1489, respectively, according to the *Escherichia coli* 16S rRNA numbering system of the International Union of Biochemistry (Brosius *et al.*, 1978; Lane *et al.*, 1985)]. PCR amplification was performed as described previously (Imhoff & Pfennig, 2001; Imhoff *et al.*, 1998) and 16S rRNA gene sequences were obtained by cycle sequencing with a SequiTherm sequencing kit (Biozym) and chain termination reaction (Sanger *et al.*, 1977) using an automated laser fluorescence sequencer (Pharmacia). The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the NCBI-BLAST search (Altschul *et al.*, 1990) and EzTaxon server (Chun *et al.*, 2007). The CLUSTAL W algorithm of MEGA 4 software was used for sequence alignments and MEGA 4 (Tamura *et al.*, 2007) software was used for phylogenetic analysis of the individual sequences. Distances were calculated by using the Kimura correction in a pairwise deletion manner (Kimura, 1980). Neighbour-joining (NJ) and minimum evolution (ME) methods in the MEGA 4 software were used to construct phylogenetic trees. Percentage support values were obtained using a bootstrap procedure.

The genomic DNA G+C content of strain JC82^T was 73.4 mol%. The results of phylogenetic analysis of the 16S rRNA gene sequence suggested that strain JC82^T belonged to the genus *Georgenia* and was closely related to all recognised species of the genus *Georgenia*: *G. muralis* 1A-C^T (96.3 % 16S rRNA gene sequence similarity), *Georgenia thermotolerans* TT02-04^T (96 %), *Georgenia daeguensis* 2C6-43^T (96 %), *G. soli* CC-NMPT-T3^T (95.9 %), *Georgenia ruanii* YIM 004^T (95.9 %) and *Georgenia halophila* YIM 93316^T (95.4 %) (NJ tree is shown as Fig. 1 and ME tree is shown as Fig. S1, available in IJSEM online). Though strain JC82^T shared relatively high sequence similarity with *G. muralis* 1A-C^T and *G. soli* CC-NMPT-T3^T, clustering occurred with *G. halophila* YIM 93316^T.

Colonies of strain JC82^T grown on nutrient agar were bright yellow, circular (2–2.5 mm in diameter), convex with entire margin. Cells of strain JC82^T were ovoid to rod-shaped, non-motile and 0.8–1.2 μ m in diameter. Lack of motility was also seen with *G. muralis* 1A-C^T, while *G. soli* CC-NMPT-T3^T cells were motile. Strain JC82^T grew over a pH range of 4–12 (optimum, 7–10). NaCl was not essential

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers are given in parentheses). Multiple alignments, distance calculations (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining method were performed by using MEGA software version 4 (Tamura *et al.*, 2007). Bootstrap values (percentage of 500 replications) are shown at the branching points. Bar, 0.01 substitutions per nucleotide position.

for growth but could be tolerated up to 5 % (w/v), with optimum growth at 2 % NaCl. Optimum growth occurred at 25–37 °C. Casein was not hydrolysed and gelatin was not liquefied. Oxidase, chitinase, lipase and urease activities were negative, but amylase and catalase activities were positive. The absence of oxidase in strain JC82^T differentiated the novel isolate from all other members of the genus *Georgenia* (Table 1). Amylase activity was optimum at pH 10 (52.2 U ml⁻¹; Fig. S2a), and at 75 °C (Fig. S2b). *G. muralis* 1A-C^T was negative for amylase activity, while *G. soli* CC-NMPT-T3^T only had activity at pH 7–8 (simultaneous comparative assays were performed on starch agar plates).

Strain JC82^T was an obligate aerobe and chemoorgano-heterotroph. Organic substrates which support growth of strain JC82^T are given in Table S1. Whole-cell fatty acid analysis of strain JC82^T revealed that anteiso-C_{15:0} was the predominant fatty acid (40.9 %), and significant proportions of iso-C_{14:0} (11.5 %), C_{14:0} (11.5 %), C_{16:0} (9.2 %), iso-C_{15:0} (6.6 %) and iso-C_{16:0} (6.3 %) were also detected.

The major quinone of strain JC82^T was menaquinone MK-8(H₄) (98 %). Polar lipids of strain JC82^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, an unknown lipid (L1) and a glycolipid (GL3) (Fig. S3). An unidentified phospholipid (PL) of *G. muralis* 1A-C^T (Altenburger *et al.*, 2002) was identified in this study as phosphatidylinositol (PI; Fig. S3), and the major cell wall polar lipids were in concurrence with the genus *Georgenia* (Li *et al.*, 2007).

The cell-wall amino acids of strain JC82^T were L-glutamine, L-alanine, D-alanine and L-lysine, which was similar to the results for *G. muralis* 1A-C^T and *G. soli* CC-NMPT-T3^T (Fig. S4) and is characteristic of the genus *Georgenia* (peptidoglycan type A4α based on lysine as diagnostic diamino acid, murein type A11.54). However, strain JC82^T could be differentiated from members of the genus *Georgenia* based on the absence of oxidase (Table 1). Furthermore, strain JC82^T exhibited differences from its closest phylogenetic relatives, *G. muralis* 1A-C^T and *G. soli* CC-NMPT-T3^T by the presence of an unidentified glycolipid (GL3; Table 1), the

Table 1. Differential characteristics between strain JC82^T and two members of the genus *Georgenia*

Strains: 1, JC82^T; 2, *G. muralis* 1A-C^T; 3, *G. soli* CC-NMPT-T3^T. All data from this study. All strains were catalase-positive, contained MK-8(H₄) as the major quinone, and L-alanine, L-glutamic acid and L-lysine in cell wall peptidoglycan. +, Positive/present; –, negative/absent; DPG, diphosphatidylglycerol; GL1–3, unidentified glycolipids; L1–2, unidentified lipids; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PL, unidentified phospholipid; ai, anteiso.

Characteristic	1	2	3
Cell morphology	Ovoid-rod	Rod-coccus	Cocci
Motility	–	–	+
Optimum growth temperature (°C)	25–37	28–37	25–30
NaCl tolerance range (% w/v)	0–5	0–7	1–3
pH range	4–12	6–9	6.5–10
Starch hydrolysis	+	–	+
Oxidase	–	+	+
Polar lipids	DPG, PG, PI, PIM, L1, GL3	DPG, PG, PI, PIM, GL1, L1	DPG, PG, PI, PIM, GL1–2, L1–2
Major fatty acid(s)	ai-C _{15:0}	ai-C _{15:0} ai-C _{15:1}	ai-C _{15:0} ai-C _{15:1}
DNA G + C content (mol%)	73.4	70.2	72

absence of anteiso-C_{15:1} fatty acid and the presence of a thermostable alkaline amylase. Strain JC82^T differs from *G. halophila* YIM93316^T in salt tolerance and the absence of an unidentified phospholipid (PL; Tang *et al.*, 2010). Therefore, on the basis of phenotypic and genotypic distinctiveness, strain JC82^T is considered to represent a novel species of the genus *Georgenia*, for which the name *Georgenia satyanarayana* sp. nov. is proposed.

Description of *Georgenia satyanarayana* sp. nov.

Georgenia satyanarayana (sa.ty.a.na.ra.ya'na.i. N.L. gen. masc. n. *satyanarayana* of Satyanarayana, named in honour of Professor T. Satyanarayana, an eminent microbiologist who significantly contributed to the knowledge of extremophiles from India).

Forms bright yellow colonies on nutrient agar. Cells are ovoid to rod-shaped, 0.8–1.2 µm in diameter. Gram-stain-positive, oxidase-negative, catalase-positive, obligate aerobe with chemorganoheterotrophic growth mode. Good growth occurs after 48h of incubation on nutrient agar at 37 °C. Growth occurs at pH 4–12 (optimum, 7–10). NaCl is not essential for growth, but growth is enhanced in the presence of NaCl (optimum 2 %, tolerated up to 5 %). Produces thermostable alkaline amylase. Utilizes a wide range of carbon sources (Biolog) including dextrin, glycogen, mannan, amygdalin, L-arabinose, cellobiose, D-fructose, D-galactose, gentiobiose, D-gluconic acid, α-D-glucose, maltose, maltotriose, D-mannose, D-ribose, salicin, D-sorbitol, trehalose, turanose, D-xylose, glycerol, sucrose, acetic acid, adenosine, 2'-deoxyadenosine, pyruvic acid methyl ester, pyruvic acid, palatinose, inosine and thymidine. D-Galacturonic acid, melezitose, D-tagatose, α-ketovaleric acid, lactamide, L-malic acid, succinamic acid, thymidine-5'-monophosphate, DL-α-glycerol phosphate, 2,3-butanediol, D-glucose 6-phosphate and uridine are utilized weakly. The peptidoglycan type is A4α, and major quinone is MK-8(H₄). Major polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and

phosphatidylinositol mannosides. An unidentified lipid (L1) and a glycolipid (GL3) are present in moderate amounts. Major (>5 %) fatty acids are anteiso-C_{15:0}, iso-C_{14:0}, C_{14:0}, C_{16:0}, iso-C_{15:0} and iso-C_{16:0}.

The type strain JC82^T (=KCTC 19802^T=NBRC 107612^T) was isolated from a sediment sample of Lonar Lake, India. The DNA G + C content of the type strain is 73.4 mol%.

Acknowledgements

We thank Professor J. Euzéby for his expert suggestion for correct species epithet and Latin etymology. E. V. V. R. thanks CSIR for SRF fellowship. We thank Dr A. B. Arun, Yenepoya Research Centre, Yenepoya University, Mangalore, India and Dr C.-C. Young, College of Agriculture and Natural Resources, Department of Soil and Environmental Sciences, National Chung Hsing University, Taichung 402, Taiwan, ROC for providing *Georgenia soli* CC-NMPT-T3^T for comparative taxonomic analysis.

References

- Altenburger, P., Kämpfer, P., Schumann, P., Vybiral, D., Lubitz, W. & Busse, H.-J. (2002). *Georgenia muralis* gen. nov., sp. nov., a novel actinobacterium isolated from a medieval wall painting. *Int J Syst Evol Microbiol* **52**, 875–881.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Anil Kumar, P., Srinivas, T. N. R., Madhu, S., Manorama, R. & Shivaji, S. (2010a). *Indibacter alkaliphilus* gen. nov., sp. nov., an alkaliphilic bacterium isolated from a haloalkaline lake. *Int J Syst Evol Microbiol* **60**, 721–726.
- Anil Kumar, P., Srinivas, T. N. R., Pavan Kumar, P., Madhu, S. & Shivaji, S. (2010b). *Nitritalea halalkaliphila* gen. nov., sp. nov., an alkaliphilic bacterium of the family 'Cyclobacteriaceae', phylum Bacteroidetes. *Int J Syst Evol Microbiol* **60**, 2320–2325.
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.
- Cappuccino, J. G. & Sherman, N. (1998). *Microbiology: a Laboratory Manual*, 5th edn. California: Benjamin/Cummings Science Publishing.

- Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.
- Hiraishi, A. & Hoshino, Y. (1984). Distribution of rhodoquinone in *Rhodospirillaceae* and its taxonomic implications. *J Gen Appl Microbiol* **30**, 435–448.
- Hiraishi, A., Hoshino, Y. & Kitamura, H. (1984). Isoprenoid quinone composition in the classification of *Rhodospirillaceae*. *J Gen Appl Microbiol* **30**, 197–210.
- Imhoff, J. F. & Pfennig, N. (2001). *Thioflavococcus mobilis* gen. nov., sp. nov., a novel purple sulfur bacterium with bacteriochlorophyll *b*. *Int J Syst Evol Microbiol* **51**, 105–110.
- Imhoff, J. F., Süling, J. & Petri, R. (1998). Phylogenetic relationships among the *Chromatiaceae*, their taxonomic reclassification and description of the new genera *Allochrochromatium*, *Halochrochromatium*, *Isochromatium*, *Marichrochromatium*, *Thiococcus*, *Thiohalocapsa* and *Thermochrochromatium*. *Int J Syst Bacteriol* **48**, 1129–1143.
- Joshi, A. A., Kanekar, P. P., Kelkar, A. S., Shouche, Y. S., Vani, A. A., Borgave, S. B. & Sarnaik, S. S. (2008). Cultivable bacterial diversity of alkaline Lonar Lake, India. *Microb Ecol* **55**, 163–172.
- Kates, M. (1972). *Techniques of Lipidology*. New York: Elsevier.
- Kates, M. (1986). *Techniques of Lipidology: Isolation, Analysis, and Identification of Lipids*. Amsterdam: Elsevier.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L. & Pace, N. R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc Natl Acad Sci U S A* **82**, 6955–6959.
- Li, W.-J., Xu, P., Schumann, P., Zhang, Y.-Q., Pukall, R., Xu, L.-H., Stackebrandt, E. & Jiang, C.-L. (2007). *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China), and emended description of the genus *Georgenia*. *Int J Syst Evol Microbiol* **57**, 1424–1428.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J Mol Biol* **3**, 208–218.
- McKerrow, J., Vagg, S., McKinney, T., Seviour, E. M., Maszenan, A. M., Brooks, P. & Seviour, R. J. (2000). A simple HPLC method for analysing diaminopimelic acid diastereomers in cell walls of Gram-positive bacteria. *Lett Appl Microbiol* **30**, 178–182.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Nelson, N. (1944). A photometric adaptation of the somogyi method for the determination of glucose. *J Biol Chem* **153**, 375–380.
- Oren, A., Duker, S. & Ritter, S. (1996). The polar lipid composition of Walsby's square bacterium. *FEMS Microbiol Lett* **138**, 135–140.
- Sanger, F., Nicklen, S. & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A* **74**, 5463–5467.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- Tang, S.-K., Wang, Y., Lou, K., Mao, P.-H., Xu, L.-H., Jiang, C.-L., Kim, C.-J. & Li, W.-J. (2009a). *Kocuria halotolerans* sp. nov., an actinobacterium isolated from a saline soil in China. *Int J Syst Evol Microbiol* **59**, 1316–1320.
- Tang, S.-K., Wang, Y., Chen, Y., Lou, K., Cao, L.-L., Xu, L.-H. & Li, W.-J. (2009b). *Zhihengliuella alba* sp. nov., and emended description of the genus *Zhihengliuella*. *Int J Syst Evol Microbiol* **59**, 2025–2031.
- Tang, S.-K., Wang, Y., Lee, J.-C., Lou, K., Park, D.-J., Kim, C.-J. & Li, W.-J. (2010). *Georgenia halophila* sp. nov., a halophilic actinobacterium isolated from a salt lake. *Int J Syst Evol Microbiol* **60**, 1317–1421.
- Tindall, B. J. (1990a). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Tindall, B. J. (1990b). A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.
- Tindall, B. J., Rosselló-Móra, R., Busse, H.-J., Ludwig, W. & Kämpfer, P. (2010). Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* **60**, 249–266.
- Wani, A. A., Surakasi, V. P., Siddharth, J., Raghavan, R. G., Patole, M. S., Ranade, D. & Shouche, Y. S. (2006). Molecular analyses of microbial diversity associated with the Lonar soda lake in India: an impact crater in a basalt area. *Res Microbiol* **157**, 928–937.
- Woo, S. G., Cui, Y., Kang, M. S., Jin, L., Kim, K. K., Lee, S.-T., Lee, M. & Park, J. (2012). *Georgenia daeguensis* sp. nov., isolated from 4-chlorophenol enrichment culture. *Int J Syst Evol Microbiol* **62**, 1703–1709.