Extremophiles (2010) 14:397–402
DOI 10.1007/s00792-010-0317-4

ORIGINAL PAPER

Zhihengliuella salsuginis sp. nov., a moderately halophilic actinobacterium from a subterranean brine

Yi-Guang Chen · Shu-Kun Tang · Yu-Qin Zhang · Zhu-Xiang Liu · Qi-Hui Chen · Jian-Wu He · Xiao-Long Cui · Wen-Jun Li

Received: 30 January 2010 / Accepted: 4 May 2010 / Published online: 20 May 2010

Abstract A novel moderately halophilic, alkaliphilic, non-motile, non-sporulating, catalase-positive, oxidase-negative, aerobic, coccus-shaped, Gram-positive bacterium, designated strain JSM 071043^T, was isolated from a subterranean brine sample collected from a salt mine in Hunan Province, China. Growth occurred with 0.5–20% (w/v) NaCl (optimum 5–10%) at pH 6.5–10.5 (optimum pH 8.5) and at 10–40°C (optimum 25–30°C). Good growth also occurred in the presence of 0.5–20% (w/v) KCl (optimum 5–8%) or 0.5–25% (w/v) $MgCl_2 \cdot 6H_2O$ (optimum 5–10%). The peptidoglycan type was A4α (L-Lys–L-Ala–L-Glu) and major cell-wall sugars were tyvelose and mannose. The major cellular fatty acids were anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$. Strain JSM 071043^T contained MK-9 and MK-8 as the predominant menaquinones and diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol as the major polar lipids. The DNA G + C content was 67.8 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain JSM 071043^T was a member of the suborder *Micrococcineae*, and was most closely related to *Zhihengliuella halotolerans* YIM 70185^T (sequence similarity 98.9%) and *Zhihengliuella alba* YIM 90734^T (98.2%), and the three strains formed a distinct branch in the phylogenetic tree. The combination of phylogenetic analysis, DNA–DNA relatedness values, phenotypic characteristics and chemotaxonomic data supports the proposal that strain JSM 071043^T represents a novel species of the genus *Zhihengliuella*, for which the name *Z. salsuginis* sp. nov. is proposed. The type strain is JSM 071043^T (= DSM 21149^T = KCTC 19466^T).

Keywords *Zhihengliuella salsuginis* sp. nov. · Halophilic · Subterranean Brine

Communicated by A. Oren.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JSM 071043^T is FJ425902.

Y.-G. Chen (✉) · Z.-X. Liu · Q.-H. Chen · J.-W. He
College of Biology and Environmental Sciences,
Jishou University, 416000 Jishou, People's Republic of China
e-mail: mchenjsu@yahoo.com.cn

Y.-G. Chen · S.-K. Tang · X.-L. Cui · W.-J. Li
The Key Laboratory for Microbial Resources of the Ministry of Education, Laboratory for Conservation and Utilization of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University, 650091 Kunming, People's Republic of China

Y.-Q. Zhang
Institute of Medicinal Biotechnology,
Chinese Academy of Medical Sciences, Peking Union Medical College, 100050 Beijing, People's Republic of China

W.-J. Li
Guangdong Key Laboratory of Marine Materia Medica,
South China Sea Institute of Oceanology, Chinese Academy of Sciences, 510301 Guangzhou, People's Republic of China

Introduction

The genus *Zhihengliuella*, belonging to the suborder *Micrococcineae* (Stackebrandt et al. 1997), was created by Zhang et al. (2007) with the description of *Z. halotolerans* as the sole recognized species of the genus. The genus has been recently emended by Tang et al. (2009) along with the proposal of *Z. alba*. The genus was defined as mesophilic, aerobic, catalase-positive, oxidase-negative, non-motile, non-sporulating, short rod-shaped, Gram-positive bacterium, having cell-wall peptidoglycan type

A4α (L-Lys–L-Ala–L-Glu), with tyvelose and mannose as the major cell-wall sugars, MK-9 and MK-10 as the predominant menaquinones, anteiso-$C_{15:0}$ and iso-$C_{15:0}$ as the major fatty acids and phosphatidylglycerol, diphosphatidylglycerol and phosphatidylinositol as the major polar lipids. The type strains of the two recognized *Zhihengliuella* species were all isolated from saline soil in north-west China. In a recent study of the microbial diversity in the ancient salt deposit of the Xiangli Salt Mine in Hunan Province, China (Chen et al. 2009), a moderately halophilic bacterium, designated strain JSM 071043^T, was isolated from a subterranean brine sample (g L^{-1}: Na^+ 81.5, Mg^{2+} 28.3, K^+ 4.5, Ca^{2+} 0.1, pH 7.6). Based on the results of the present polyphasic taxonomic study, this strain was proposed to represent a novel species of the genus *Zhihengliuella*.

Materials and methods

Strains and culture conditions

Strain JSM 071043^T was isolated from a subterranean brine sample by plating 1:10 serial dilutions of the sample on marine agar 2216 (MA; Difco) supplemented with 5% (w/v) NaCl (hereafter MA5) at 30°C for 2 weeks. After primary isolation, the strain was purified by repeated streaking and subculturing on MA5 plates and examining the cultures by light microscopy. It was maintained either as serial transfers on MA5 slants, or lyophilized cultures at 4°C, or deep-frozen in 20% (v/v) glycerol at −80°C. For comparison, two type strains, *Z. halotolerans* YIM 70185^T and *Z. alba* YIM 90734^T, were obtained from the Yunnan Institute of Microbiology (YIM, 650091 Kunming, China). Unless indicated otherwise, morphological, physiological, molecular and chemotaxonomic studies were performed with cells grown on MA5 (pH 8.5) at 28°C.

Phenotypic characterization

Cell morphology was examined by using light microscopy (model DM3000; Leica). The Gram staining and the KOH lysis test were carried out according to Doetsch (1981) and Gregersen (1978), respectively. Growth in the absence of salt was investigated on nutrient agar (NA) prepared according to the formula of Atlas and Parks (1993) except that NaCl was excluded. Tolerance of salt was tested on NA in different salt (NaCl, KCl, $MgCl_2{\cdot}6H_2O$) concentrations [0.1 and 0.5% (w/v), and 1–30% (w/v) at increments of 1%]. Growth was tested at various temperatures (4, 5–55°C, at increments of 5°C) and at different pH (5.0–11.0, at increments of 0.5 pH units) on MA5 as well as in nutrient broth (Atlas and Parks 1993) supplemented with 7% (w/v) NaCl. For pH tolerance experiments, the buffer solutions described by Chen et al. (2004) were used. Growth under anaerobic conditions was determined on MA5 supplemented with 0.5% (w/v) glucose and with or without 0.1% (w/v) nitrate by using the GasPak Anaerobic Systems (BBL) according to the manufacturer's instructions. Methyl red and Voges–Proskauer tests and determination of hydrolysis of aesculin, indole and H_2S production, nitrate and nitrite reduction, and lysine decarboxylase, phenylalanine deaminase and ornithine decarboxylase were assessed as recommended by Smibert and Krieg (1994). Hydrolysis of casein, cellulose, chitin, DNA, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, urea and xanthine was determined as described by Cowan and Steel (1965). Determination of acid production from carbohydrates and utilization of carbon and nitrogen sources was performed as recommended by Ventosa et al. (1982). Observation of motility and tests of catalase and oxidase activities were determined as described previously (Chen et al. 2007). Other enzymic activities were also assayed by using API ZYM strips (bioMérieux) according to the manufacturer's instructions with 7.5% (w/v) NaCl.

Determination of 16S rRNA gene sequence, phylogenetic analysis and DNA–DNA hybridization

The 16S rRNA gene sequence was amplified by PCR and sequenced as described by Cui et al. (2001). Pairwise sequence similarities were calculated using a global alignment algorithm, implemented at the EzTaxon server (Chun et al. 2007). Phylogenetic analysis was performed by using the software package MEGA version 3.1 (Kumar et al. 2004) after multiple alignment of sequence data by CLUSTAL_X (Thompson et al. 1997). Distances were calculated using distance options according to Kimura's two-parameter model (Kimura 1980) and clustering was performed with the neighbour-joining method (Saitou and Nei 1987). Maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Kluge and Farris 1969) trees were generated by using the treeing algorithms contained in the PHYLIP package (Felsenstein 2002). Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by means of 1,000 resamplings (Felsenstein 1985). DNA–DNA hybridization experiments were carried out using the optical renaturation method (De Ley et al. 1970; Huß et al. 1983; Jahnke 1992).

Chemotaxonomic characterization

Purified cell-wall preparations were obtained and hydrolyzed as described by Schleifer and Kandler (1972). Sugars, amino acids and peptides in cell-wall hydrolysates

were analyzed by HPLC as described by Tang et al. (2009). Polar lipids were extracted according to the method of Minnikin et al. (1984) and identified by two-dimensional TLC and spraying with appropriate detection reagents (Collins and Jones 1980). Isoprenoid quinones were analyzed by means of HPLC as described by Groth et al. (1996). Fatty acid compositions were determined according to Sasser (1990) by using the Microbial Identification System (Microbial ID). Genomic DNA was isolated according to Hopwood et al. (1985) and the G + C content was determined using the HPLC method (Mesbah et al. 1989).

Results and discussion

Phenotypic characteristics

Strain JSM 071043^T was moderately halophilic and alkaliphilic, growth occurring in the presence of 0.5–20% (w/v) NaCl (optimum 5–10%), at pH 6.5–10.5 (optimum pH 8.5) and at 10–40°C (optimum 25–30°C). Good growth also occurred in the presence of 0.5–20% (w/v) KCl (optimum 5–8%) or 0.5–25% (w/v) $MgCl_2 \cdot 6H_2O$ (optimum 5–10%). Colonies were light yellow-pigmented, circular, somewhat convex and non-translucent with glistening surfaces and entire margins and 2–3 mm in diameter after incubation for 3–5 days at 28°C on MA5. Detailed phenotypic properties that differentiate strain JSM 071043^T from recognized *Zhihengliuella* species are summarized in Table 1 and also mentioned in the species description below.

Phylogenetic analysis based on 16S rRNA gene sequence comparison and DNA–DNA relatedness

The almost-complete 16S rRNA gene sequence (1,421 bp) of the organism was determined. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain JSM 071043^T was a member of the suborder *Micrococcineae* and was most closely related to the type strains of the two recognized species of the genus *Zhihengliuella*, *Z. halotolerans* YIM 70185^T (sequence similarity 98.9%) and *Z. alba* YIM 90734^T (98.2%), and the three strains formed a distinct clade supported by a significant bootstrap resampling value (100%) in the phylogenetic tree (Fig. 1). The topology was similar to those of the phylogenetic trees constructed by using maximum-likelihood and maximum-parsimony methods (not shown). Levels of DNA–DNA relatedness between strain JSM 071043^T and the type strains of *Z. halotolerans* and *Z. alba* were 30.9 and 20.5%, respectively, values that are well below the threshold value (70%) recommended by Wayne et al. (1987) for assigning strains to the same species. Therefore, it would appear that,

Table 1 Characteristics used to distinguish strain JSM 071043^T from recognized *Zhihengliuella* species

Characteristic	1	2	3
Colony pigmentation	Light yellow	Pale yellow	White
Cell morphology	Cocci	Short rod	Short rod
NaCl range (%, w/v)	0.5–20	0–25	0–15
NaCl optimum (%, w/v)	5–10	10	5
Temperature range (°C)	10–40	4–45	4–45
Temperature optimum (°C)	25–30	25–30	30
pH range	6.5–10.5	6.0–10.0	6.0–9.5
pH optimum	8.5	8.5	7.5
Hydrolysis of			
Aesculin	−	+	+
Starch	+	+	−
Tween 20	+	+	−
Acid production from			
L-Arabinose	−	+	−
Cellobiose	+	−	−
Glycogen	+	+	−
Lactose	−	+	+
D-Mannose	−	+	−
Raffinose	−	+	−
Trehalose	−	−	+
Adonitol	−	+	−
Glycerol	+	+	−
myo-Inositol	−	+	−
D-Mannitol	−	+	+
Salicin	−	+	−
D-Sorbitol	−	+	−
Xylitol	+	+	−
Utilization			
L-Arabinose	−	+	−
Cellobiose	−	−	+
D-Fructose	−	+	+
Lactose	−	−	+
D-Mannose	−	+	+
Melibiose	−	+	−
Raffinose	+	−	−
D-Ribose	+	−	−
Trehalose	−	+	+
myo-Inositol	−	+	+
D-Mannitol	+	−	−
D-Sorbitol	−	+	−
Salicin	−	+	−
Acetate	+	−	−
L-Alanine	−	+	−
L-Arsparagine	−	−	+
L-Serine	−	−	+

Table 1 continued

Characteristic	1	2	3
Enzyme activity (API ZYM)			
Alkaline phosphatase	+	+	−
Lipase (C14)	−	+	−
Valine arylamidase	−	−	+
Cystine arylamidase	−	−	+
Trypsin	+	−	+
α-Chymotrypsin	+	−	+
Acid phosphatase	−	−	+
Naphthol-AS-BI-phosphohydrolase	−	−	+
β-Glucuronidase	+	−	−
N-Acetyl-β-glucosaminidase	+	−	−
α-Glucosidase	−	−	+
α-Fucosidase	+	−	−
Predominant menaquinones[a]	9, 8	9, 10	9, 10
DNA G + C content (mol%)[a]	67.8	66.5	70.3

Strains: *1 Z. salsuginis* sp. nov. JSM 071043^T; *2 Z. halotolerans* YIM 70185^T; *3 Z. alba* YIM 90734^T

+ Positive, − negative

All strains are non-motile, non-sporulating, catalase-positive and oxidase-negative, aerobic, Gram-positive actinobacteria. All strains are positive for activity of esterase (C4), esterase lipase (C8), β-glucosidase and hydrolysis of gelatin and Tweens 40 and 80, but negative for nitrate and nitrite reduction, H_2S and indole production, methyl red and Voges–Proskauer test and activity of leucine arylamidase, α-galactosidase, β-galactosidase and α-mannosidase. All strains produce acid from D-fructose, D-glucose, maltose, L-rhamnose, D-ribose, starch, sucrose and D-xylose, but not from dulcitol, D-galactose, inulin, melibiose and melezitose. All strains utilize the following substrates as sole carbon or nitrogen and energy sources: dextrin, D-glucose, maltose, sucrose, glycerol, gluconate and L-glutamic acid, but the following are not utilized: D-galactose, glycogen, inulin, melezitose, L-rhamnose, D-xylose, adonitol, butyrate, citrate, formate, fumarate, malate, malonate, propionate, succinate, acetamide, L-arginine, L-glycine, L-histidine, hydroxy-L-proline, L-isoleucine, L-leucine, L-methionine, L-proline and L-valine

[a] Data for strains *Z. halotolerans* YIM 70185^T and *Z. alba* YIM 90734^T were from Zhang et al. (2007) and Tang et al. (2009), respectively. Other data were from this study

on the basis of the phylogenetic and DNA–DNA hybridization data, strain JSM 071043^T represents a new species of the genus *Zhihengliuella* according to accepted criteria (Wayne et al. 1987; Stackebrandt and Goebel 1994).

Chemotaxonomic characteristics and DNA base composition

Chemotaxonomic data for strain JSM 071043^T were consistent with the assignment of the strain to the genus *Zhihengliuella*. The peptidoglycan type was A4α (L-Lys–L-Ala–L-Glu) and major cell-wall sugars were tyvelose and mannose, which are characteristic of the genus *Zhihengliuella* (Zhang et al. 2007; Tang et al. 2009). MK-9 (56.1%) and MK-8 (26.4%) were detected as the major menaquinones, with MK-9 (H_2) (3.0%), MK-9 (H_4) (5.7%) and MK-10 (8.8%) present in minor amounts. The polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, an unknown phospholipid and an unknown glycolipid. The major fatty acids (>10% of the total) of strain JSM 071043^T were anteiso-$C_{15:0}$ (56.1%), iso-$C_{16:0}$ (17.0%) and anteiso-$C_{17:0}$ (12.4%); iso-$C_{14:0}$ (3.0%), iso-$C_{15:0}$ (3.9%), $C_{16:0}$ (4.1%), iso-$C_{17:0}$ (0.5%), $C_{18:0}$ (0.5%) and $C_{18:1}$ 2-OH (0.5%) were present in minor amounts. This fatty acid profile was similar to those of recognized *Zhihengliuella* species, although there were differences in the proportions of some components (Zhang et al. 2007; Tang et al. 2009). The DNA G + C content of strain JSM 071043^T was 67.8 mol%.

Taxonomic conclusion

The results of the phylogenetic analysis and of morphological and chemotaxonomic investigations supported the affiliation of strain JSM 071043^T to the genus *Zhihengliuella*. However, the novel strain could be clearly distinguished from the two recognized *Zhihengliuella* species by differences in several phenotypic and chemotaxonomic characteristics, including cell morphology, growth condition, utilization of and acid production from several substrates, activity of some enzymes and megaquinone composition (Table 1). Although strain JSM 071043^T exhibited comparatively high 16S rRNA gene sequence similarity with *Arthrobacter oryzae* (97.5%; Kageyama et al. 2008) and some other related *Arthrobacter* species (<96.9%), the new isolate, as well as the two recognized *Zhihengliuella* species, differed clearly from related *Arthrobacter* species by sufficient taxonomic markers as discussed previously (Zhang et al. 2007; Tang et al. 2009). In conclusion, the results of phylogenetic analysis based on 16S rRNA gene sequences and DNA–DNA hybridization experiments and the phenotypic and chemotaxonomic data presented here supported the proposal of strain JSM 071043^T representing a novel species of the genus *Zhihengliuella*, for which the name *Z. salsuginis* sp. nov. is proposed.

Description of *Z. salsuginis* sp. nov.

Z. salsuginis (sal.su'gi.nis. L. gen. n. *salsuginis* of/from brine)

Cells are non-motile, non-sporulating, aerobic, Gram-positive, coccoid and about 0.6–0.9 mm in diameter and form pairs, tetrads and clusters. Oxidase-negative and catalase-positive. Colonies are light yellow-pigmented,

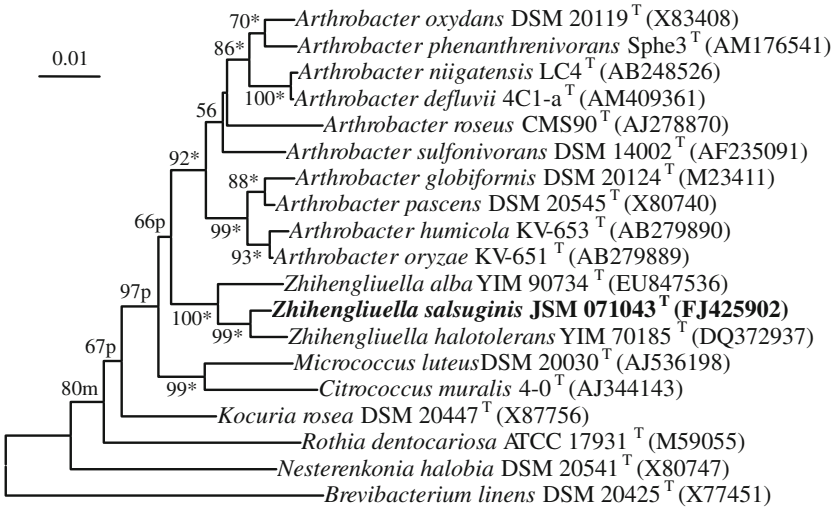

Fig. 1 Phylogenetic tree showing the phylogenetic positions of strain JSM 071043^T and related taxa based on 16S rRNA gene sequence analysis constructed by using the neighbour-joining method. Labels *m* or *p* indicate branches that are also found with the maximum-likelihood (Felsenstein 1981) or parsimony (Kluge and Farris 1969) algorithms, respectively; *asterisks* indicate branches that are recovered with all three methods. *Numbers at nodes* indicate bootstrap percentages (>50%) based on a neighbour-joining analysis of 1,000 resampled datasets. The *scale bar* indicates 0.01 substitutions per nucleotide position

circular, somewhat convex and non-translucent with glistening surfaces and entire margins and 2–3 mm in diameter. Moderately halophilic, alkaliphilic and mesophilic, growth occurring with 0.5–20% (w/v) NaCl (optimum 5–10%), at pH 6.5–10.5 (optimum pH 8.5) and at 10–40°C (optimum 25–30°C). Good growth also occurs in the presence of 0.5–20% (w/v) KCl (optimum 5–8%) or 0.5–25% (w/v) $MgCl_2{\cdot}6H_2O$ (optimum 5–10%). Positive for hydrolysis of gelatin, starch, Tweens 20, 40, 60 and 80, but negative for nitrate and nitrite reduction, H_2S and indole production, methyl red and Voges-Proskauer test and hydrolysis of aesculin, casein, cellulose, chitin, DNA, hypoxanthine, urea and xanthine. Produces acid from cellubiose, D-fructose, D-glucose, glycerol, glycogen, maltose, L-rhamnose, D-ribose, starch, sucrose, xylitol and D-xylose, but not from L-arabinose, D-galactose, inulin, lactose, D-mannose, melibiose, melezitose, raffinose, trehalose, adonitol, dulcitol, *myo*-inositol, D-mannitol, salicin and D-sorbitol. The following compounds are utilized as sole sources of carbon and energy or sole sources of carbon, nitrogen and energy: dextrin, D-glucose, maltose, raffinose, D-ribose, sucrose, glycerol, D-mannitol, acetate, gluconate and L-glutamic acid; the following are not utilized: L-arabinose, cellobiose, D-fructose, D-galactose, glycogen, inulin, lactose, D-mannose, melezitose, melibiose, L-rhamnose, trehalose, D-xylose, adonitol, *myo*-inositol, D-sorbitol, salicin, butyrate, citrate, formate, fumarate, malate, malonate, propionate, succinate, acetamide, L-alanine, L-arginine, L-asparagine, L-glycine, L-histidine, hydroxy-L-proline, L-isoleucine, L-leucine, L-methionine, L-proline, L-serine and L-valine. Constitutive enzymes expressed are *N*-acetyl-β-glucosaminidase, alkaline phosphatase, α-chymotrypsin, esterase (C4), α-fucosidase, β-glucosidase, β-glucuronidase, lipase (C8) and trypsin; acid phosphatase, cystine arylamidase, α- and β-galactosidase, α-glucosidase, leucine arylamidase, lipase (C14), lysine decarboxylase, α-mannosidase, naphthol-AS-BI-phosphohydrolase, ornithine decarboxylase, phenylalanine deaminase or valine arylamidase are not observed. The peptidoglycan type is A4α (L-Lys–L-Ala–L-Glu) and major cell-wall sugars are tyvelose and mannose. Possesses MK-9 and MK-8 as the predominant menaquinones and diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol as the major polar lipids. Major cellular fatty acids (>10% of the total) are anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$. The DNA G + C content of the type strain is 67.8 mol% (HPLC method).

The type strain, JSM 071043^T (= DSM 21149^T = KCTC 19466^T), was isolated from a subterranean brine sample collected from the Xiangli Salt Mine in Hunan Province, China.

Acknowledgments This work was supported by grants from the National Basic Research Program of China (2010CB833800), National Natural Science Foundation of China (NSFC) (30970007) and International Cooperation Research Program of Yunnan Province (2009AC017). We are grateful to Mr. Ke Huang for his excellent technical assistance.

References

Atlas RM, Parks LC (1993) Handbook of microbiological media. CRC Press, Boca Raton, pp 666–672

Chen HH, Li WJ, Tang SK, Kroppenstedt RM, Stackebrandt E, Xu LH, Jiang CL (2004) *Corynebacterium halotolerans* sp. nov., isolated from saline soil in the west of China. Int J Syst Evol Microbiol 54:779–782

Chen YG, Cui XL, Pukall R, Li HM, Yang YL, Xu LH, Wen ML, Peng Q, Jiang CL (2007) *Salinicoccus kunmingensis* sp. nov., a moderately halophilic bacterium isolated from a salt mine in Yunnan, south-west China. Int J Syst Evol Microbiol 57:2327–2332

Chen YG, Zhang YQ, Liu ZX, Zhuang DC, Klenk HP, Tang SK, Cui XL, Li WJ (2009) *Halobacillus salsuginis* sp. nov., a moderately halophilic bacterium from a subterranean brine. Int J Syst Evol Microbiol 59:2505–2509

Chun J, Lee JH, Jung Y, Kim M, Kim S, Kim BK, Lim YW (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

Collins MD, Jones D (1980) Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2, 4-diaminobutyric acid. J Appl Bacteriol 48:459–470

Cowan ST, Steel KJ (1965) Manual for the identification of medical bacteria. Cambridge University Press, London

Cui XL, Mao PH, Zeng M, Li WJ, Zhang LP, Xu LH, Jiang CL (2001) *Streptomonospora salina* gen. nov., sp. nov., a new member of the family *Nocardiopsaceae*. Int J Syst Evol Microbiol 51:357–363

De Ley J, Cattoir H, Reynaerts A (1970) The quantitative measurement of DNA hybridization from renaturation rates. Eur J Biochem 12:133–142

Doetsch RN (1981) Determinative methods of light microscopy. In: Gerhardt P, Murray RGE, Costilow RN, Nester EW, Wood WA, Krieg NR, Phillips GH (eds) Manual of methods for general bacteriology. American Society for Microbiology, Washington, pp 21–33

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Felsenstein J (2002) PHYLIP (phylogeny inference package), version 3.6a. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA

Gregersen T (1978) Rapid method for distinction of Gram-negative from Gram-positive bacteria. Eur J Appl Microbiol Biotechnol 5:123–127

Groth I, Schumann P, Weiss N, Martin K, Rainey FA (1996) *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. Int J Syst Bacteriol 46:234–239

Hopwood DA, Bibb MJ, Chater KF, Kieser T, Bruton CJ, Kieser HM, Lydiate DJ, Smith CP, Ward JM (1985) Preparation of chromosomal, plasmid and phage DNA. In: Hopwood DA, Bibb MJ, Chater KF, Kieser T, Bruton CJ, Kieser HM, Lydiate DJ, Smith CP, Ward JM, Schrempf H (eds) Genetic manipulation of *Streptomyces*: a laboratory manual. F. Crowe and Sons, Norwich, pp 79–80

Huß VAR, Festl H, Schleifer KH (1983) Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. Syst Appl Microbiol 4:184–192

Jahnke KD (1992) BASIC computer program for evaluation of spectroscopic DNA renaturation data from Gilford System 2600 spectrophotometer on a PC/XT/AT type personal computer. J Microbiol Methods 15:61–73

Kageyama A, Takahashi Y, Morisaki K, Omura S (2008) *Arthrobacter oryzae* sp. nov. and *Arthrobacter humicola* sp. nov. Int J Syst Evol Microbiol 58:53–56

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

Kluge AG, Farris FS (1969) Quantitative phyletics and the evolution of anurans. Syst Zool 18:1–32

Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163

Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. Int J Syst Bacteriol 39:159–167

Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101, MIDI Inc, Newark, DE

Schleifer KH, Kandler O (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol Rev 36:407–477

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, pp 607–654

Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol 44:846–849

Stackebrandt E, Rainey FA, Ward-Rainey NL (1997) Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. Int J Syst Bacteriol 47:479–491

Tang ST, Wang Y, Chen Y, Lou K, Cao LL, Xu LH, Li WJ (2009) *Zhihengliuella alba* sp. nov., and emended description of the genus *Zhihengliuella*. Int J Syst Evol Microbiol 59:2025–2032

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Ventosa A, Quesada E, Rodriguez-Valera F, Ruiz-Berraquero F, Ramos-Cormenzana A (1982) Numerical taxonomy of moderately halophilic Gram-negative rods. J Gen Microbiol 128:1959–1968

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE et al (1987) International committee on systematic bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464

Zhang YQ, Yu LY, Liu HY, Zhang YQ, Xu LH, Stackebrandt E, Jiang CL, Li WJ (2007) *Zhihengliuella halotolerans* gen. nov., sp. nov. a novel member of the family *Micrococcaceae* from a saline soil in China. Int J Syst Evol Microbiol 57:1018–1023