J. Gen. Appl. Microbiol., **60**, 101−105 (2014)
doi 10.2323/jgam.60.101

jGAM

Full Paper

Bacillus shacheensis sp. nov., a moderately halophilic bacterium isolated from a saline-alkali soil

(Received December 20, 2013; Accepted April 3, 2014)

Zuchao Lei,[1,2,#] Peng Qiu,[2,#] Renyuan Ye,[2] Jiewei Tian,[2] Yang Liu,[2] Lei Wang,[2] Shu-Kun Tang,[3] Wen-Jun Li,[3] and Yongqiang Tian[1,*]

[1] *Key Laboratory of Leather Chemistry and Engineering, Ministry of Education and College of Light Industry, Textile & Food Engineering, Sichuan University, Chengdu, 610065, China*
[2] *Department of Pharmaceutical and Biological Engineering, School of Chemical Engineering, Sichuan University, Chengdu, Sichuan 610065, China*
[3] *The Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan, 650091, People's Republic of China*

A moderately halophilic bacterium, strain HNA-14^T, was isolated from a saline-alkali soil sample collected in Shache County, Xinjiang Province. On the basis of the polyphasic taxonomic data, the isolate was considered to be a member of the genus *Bacillus*. The organism grew optimally at 30°C and pH 8.0. It was moderately halophilic and its optimum growth occurred at 5−10% NaCl. The diamino acid found in the cell-wall peptidoglycan was *meso*-diaminopimelic acid and the predominant menaquinone was MK-7. The major cellular fatty acids were *anteiso*-$C_{15:0}$ and *iso*-$C_{15:0}$ and the polar lipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides and two unknown phospholipids. The G+C content of the genomic DNA was 48.6 mol%. Strain HNA-14^T exhibited a low 16S rRNA gene sequence similarity of 96% with its nearest neighbors [*Bacillus clausii* KSM-K16 (96.5%), *Bacillus xiaoxiensis* DSM 21943^T(96.2%), *Bacillus clausii* DSM 8716^T (96.1%), *Bacillus patagoniensis* PAT05^T (96.1%), *Bacillus lehensis* MLB-2^T (96.0%), *Bacillus oshimensis* K11^T (95.9%) and *Bacillus hunanensis* DSM 23008^T (95.8%)] and the phenotypic characteristics indicate that strain HNA-14^T can be distinguished from them. Therefore, a novel species of the genus *Bacillus*, *Bacillus shacheensis* sp. nov. (type strain, HNA-14^T=KCTC 33145=DSM 26902) is proposed.

Key Words: *Bacillus shacheensis* sp. nov.; moderately halophilic; saline-alkali soil; Xinjiang Province

Introduction

The genus *Bacillus* was first described by Cohn in 1872. Early in the history of microbiology, members of the *Bacillus* genus (266 species) were found ubiquitously, and it is one of the genera with the largest 16S diversity and environmental diversity (Logan et al., 2007). Many halophilic *Bacilli* were isolated from a wide range of habitats recently, such as the intestinal tract of an earthworm (Hong et al., 2012), foods (Seiler et al., 2012), a hypersaline lake (Bagheri et al., 2012), soybean root (Zhang et al., 2012) and non-saline forest soil (Chen et al., 2011c). In this paper, we report on taxonomic characterization of a moderately halophilic, Gram-staining-positive and spore-forming bacterial strain, HNA-14^T, which was isolated from a saline-alkali soil sample collected in Shache County, Xinjiang Province, north-west China. On the basis of phenotypic characteristics, chemotaxonomic data, phylogenetic analysis and 16S rRNA data, the isolate is considered to represent a novel species of the genus *Bacillus*.

Materials and Methods

Strains and culture conditions. Strain HNA-14^T was isolated from a saline-alkali soil sample collected in Shache County, Xinjiang Province by the serial dilution plating

*Corresponding author: YongqiangTian, College of Light Industry, Textile and Food Engineering, Sichuan University, Chengdu 610065, PR China.
Tel: +86 (28) 85405237 Fax: +86 (28) 85405237 E-mail: yqtian@scu.edu.cn
#These authors contributed equally to this work.

None of the authors of this manuscript has any financial or personal relationship with other people or organizations that could inappropriately influence their work.

method, using the HNA enrichment medium (casein peptone 7.5 g, yeast extract 10 g, NaCl 150 g, H_2O 1 L, pH 8.0) at 30°C. Subculturing was performed on trypticase soy broth (TSB) supplemented with 10% (w/v) NaCl at 30°C for 48 h and the bacterial isolate was maintained as glycerol stock at −80°C. Reference strains used were *Bacillus clausii* DSM 8716^T, *Bacillus xiaoxiensis* DSM 21943^T and *Bacillus hunanensis* DSM 23008^T, obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany).

Phenotypic characterization. Colony morphology was investigated by observing the growth of the strain on TSB supplemented with 10% (w/v) NaCl at 30°C for 48 h. Cell morphology was examined by using light microscopy (E100; Nikon) at 1,000× and scanning electron microscopy (JSM-7500F; JEOL). Motility was checked according to the method described by Skerman (1967). The Gram reaction was carried out following Gerhardt et al. (1981). Growth was tested at different temperatures (0−75°C, in increments of 5°C), NaCl concentrations [0−30% (w/v) in increments of 1%] and pH (5.0−11.0, in increments of 0.5 units) (Cowan and Steel, 1965). The pH was adjusted using appropriate biological buffers [Chen et al. (2007)]. Hydrolysis of gelatin, urease, starch, and carbon; nitrogen utilization; esterase, methyl red and Voges-Proskauer tests; catalase and oxidase; production of H_2S and indole; and reduction of nitrate were determined as described by Cowan and Steel (1965) and Smibert and Krieg (1994). Tests for utilization of different substrates were performed with a BIOLOG-GNIII system and other enzymatic activities were assayed by using API ZYM strips according to the manufacturer's instructions with 5% (w/v) NaCl.

Chemotaxonomic characterization. After being cultivated in trypticase soy broth for 24 h at 30°C, the strains were prepared as freeze-dried cells for the chemotaxonomic analyses except for the fatty acid study. The amino acids were analyzed using TLC as described by Schleifer (1985) and Schleifer and Kandler (1972). Polar lipids were extracted, examined using two-dimensional TLC and identified as described by Minnikin et al. (1984). Menaquinones were extracted and separated by HPLC following by the methods of Minnikin et al. (1984) and Kroppenstedt (1982). The cellular fatty acid analysis was performed using the strains grown on TSB at 30°C for 2 days as described by Pandey et al. (2002) according to the MIDI Sherlock Microbial Identification System (MIDI, Inc.).

Determination of 16S rRNA gene sequence, phylogenetic analysis. The genomic DNA was isolated and purified with a genomic DNA isolation kit (DNeasy 96 Blood & Tissue Kit 4, Qiagen, Hilden, Germany) and the G+C content was determined using the method of Mesbah et al. (1989). After being amplified by PCR, the 16S rRNA gene was sequenced as described as Ghosh et al. (2006). The multiple alignment of sequence data was performed by CLUSTAL X (Thompson et al., 1997). Phylogenetic analysis was conducted using maximum likelihood (Tamura et al., 2011) in MEGA 5.10. The published names of closely related taxa and the data on the pairwise sequence similarities were retrieved at the EzTaxon server (Chun et al., 2007) and the distances between the sequences were calculated using the method of Jukes and Cantor (1969). The topology of the phylogenetic tree was evaluated by means of 1,000 resamplings (Felsenstein, 1985).

Results and Discussion

Phenotypic characteristics

Strain HNA-14^T is a moderarely halophilic, obligate aerobic, Gram-staining-positive, motile and rod-shaped (0.5−0.8 × 1.0−1.5 μm) bacterium (Fig. 1). The ellipsoidal spores lie subterminally, and sporangia are unswollen. Colonies are circular, smooth and yellowish. Growth occurs with 0.5−20% (w/v) NaCl (optimum 5−10%), at pH 7.0−10 (optimum pH 8.0) and at 5−40°C (optimum 25−30°C). Hydrolysis of casein, gelatin, starch and Tween 40 is observed, but Tween 20, 60 and 80 are not hydrolyzed. Oxidase and catalase reactions are positive, but negative for urease, egg yolk reaction, methyl red, H_2S, Voges-Proskauer and indole production tests. Nitrate is not reduced to nitrite. Detailed phenotypic properties that differentiate strain HNA-14^T from the three closely related type strains of species in the genus *Bacillus* are presented in Table 1 and also mentioned in the species description below.

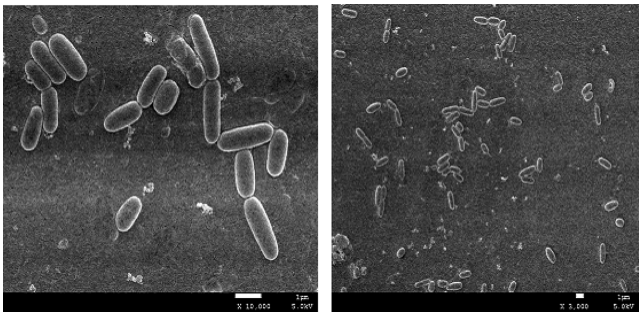

Fig. 1. Scanning electron micrographs showing strain HNA-14^T short rod sparse spores.

The organism was grown on trypticase soy broth (TSB) supplemented with 10% (w/v) NaCl at 28°C for 3 days.

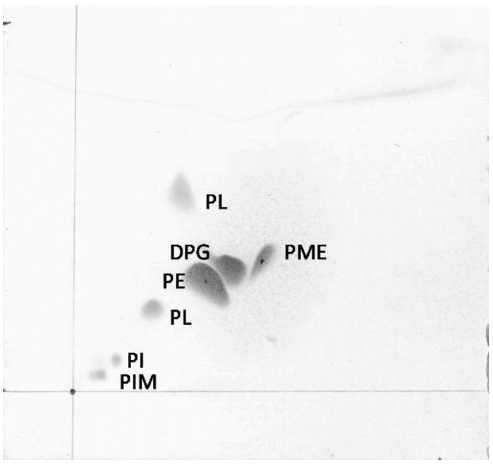

Fig. 2. Two-dimensional TLC of the polar lipids of strain HNA-14^T.

DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIM, phosphatidylinositolmannosides; PL, phospholipid; PME, phosphatidylmonomethylethanolamine.

Table 1. Characteristics used to distinguish strain HNA-14^T from the type strains of phylogenetically related species of the genus *Bacillus*.

Characteristic	1	2	3	4
Colony color	Yellowish	Yellow	Cream-white	Yellow
Spore location	Subterminal	Central to subterminal	Subterminal to central	Central
Sporangium	Unswollen	Slightly swollen	Slightly swollen	Unswollen
Anaerobic growth	−	+	+	−
DNA G+C content (mol%)	48.6	40.1	41.7−43.5	40.9
Oxidase	+	−	+	+
Nitrate reduction	−	+	+	−
Hydrolysis of:				
Tween 20	−	+	−	+
Tween 40	w	−	w	+
Tween 60	−	−	−	+
Tween 80	−	−	w	−
Growth conditions:				
NaCl range (%, w/v)	0.5−20	0.5−20	0.5−10	0.5−15
NaCl optimum (%, w/v)	5.0−10	2−4	2−5	2−4
pH range	7.0−10	6.0−10.5	7−10.5	6.5−10.5
pH optimum	7.5−8.0	8	9	7.5−8.5
Temperature range (°C)	5−40	5−40	15−50	5−40
Temperature optimum (°C)	25−30	25−30	40	30
Utilization of:				
N-acetylglucosamine	−	−	+	+
Dextrin	−	−	+	+
D-Fructose	−	−	+	+
D-Glucose	−	+	+	+
Maltose	+	−	w	+
D-Mannose	−	+	+	+
Sucrose	w	−	+	+
D-Mannitol	−	−	+	+
Lactose	w	−	+	−
Melibiose	−	−	+	−
Raffinose	w	−	+	−
L-Rhamnose	−	−	+	−
D-Salicin	w	+	+	−
Trehalose	w	−	w	−
D-Arabitol	w	−	+	−
Glycerol	−	+	+	−
myo-Inositol	w	−	+	−
D-Sorbitol	w	−	+	−
Butyrate	w	−	+	−
L-Glutamic acid	−	−	+	−
Enzyme:				
α-Chymotrypsin	−	+	−	+
Lipase(C14)	−	−	−	+
α-Mannosidase	−	−	−	+
β-Glucuronidase	−	−	+	−
Leucine arylamidase	−	+	−	−
Naphthol-AS-BI-phosphohydrolase	+	+	+	−
Trypsin	−	−	−	−
Valine arylamidase	+	−	−	−
Alkaline phosphatase	−	+	+	+
Acid phosphatase	+	−	+	+

Strains: 1, *Bacillus shacheensis* sp. nov.; 2, *B. xiaoxiensis* DSM 21943^T; 3, *B. clausii* DSM 8716^T; 4, *B. hunanensis* DSM 23008^T . All strains are endospore-forming, Gram-stain-positive rods. All strains are positive for catalase, esterase(C4) , esterase lipase(C8) activity and hydrolysis of casein, gelatin, and starch. All strains are negative for: egg yolk reaction; indole and H_2S production; methyl red and Voges-Proskauer tests and cystine arylamidase, α-fucosidase, α- and β-galactosidase, α- and β-glucosidase, *N*-acetyl-β-glucosaminidase and trypsin. All data were obtained from this study unless indicated otherwise. +, positive; −, negative; w, weak growth.

Data for the type strains of *B. xiaoxiensis* and *B. hunanensis* were obtained from Yi-Guang Chen et al. (2011).

Chemotaxonomic characteristics and DNA base composition

The diamino acid found in the cell-wall peptidoglycan of HNA-14^T was *meso*-diaminopimelic acid and the predominant menaquinone was MK-7. The major cellular fatty acids were *anteiso*-$C_{15:0}$ (43.16%), *iso*-$C_{15:0}$ (11.89%), *iso*-$C_{16:0}$ (7.65%) and *anteiso*-$C_{17:0}$ (7.45%). The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides and two unknown phospholipids (Fig. 2). The G+C content of the genomic DNA was 48.6 mol%, which falls within the defined range (32−69 mol%) accepted for *Bacillus* species (Fritze et al., 1990). The chemotaxonomic properties were all the typical characteristics of the genus *Bacillus*.

Phylogenetic analysis based on 16S rRNA gene sequence comparison

The complete sequence (1,570 bases) of the 16S rRNA

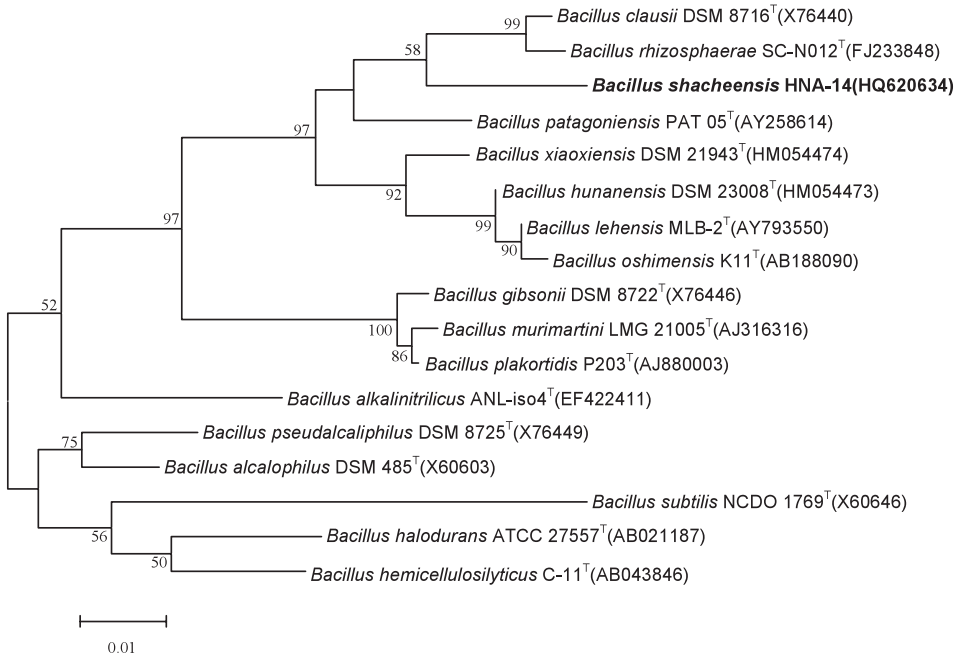

Fig. 3. Phylogenetic tree showing the phylogenetic positions of strain HNA-14^T and related taxa based on 16S rRNA gene sequence analysis constructed using the maximum likelihood method.

Numbers at nodes are bootstrap percentages (>50%) based on a maximum-likelihood analysis of 1,000 resampled datasets. Bar: 1 substitution per 100 nucleotides.

gene of strain HNA-14^T was determined and compared with those of closely related taxa retrieved from the Eztaxon database. The phylogenetic tree was constructed using the maximum-likelihood method; in the phylogenetic tree, strain HNA-14^T formed a distinct lineage within the genus *Bacillus*. Pairwise sequence analysis revealed that the highest sequence similarity was with *Bacillus clausii* KSM-K16 (96.481%, Nielsen et al., 1995), followed by *Bacillus xiaoxiensis* DSM 21943^T (96.180%, Chen et al., 2011b), *Bacillus clausii* DSM 8716^T (96.086%, Nielsen et al., 1995), *Bacillus patagoniensis* PAT 05^T (96.077%, Olivera et al., 2005), *Bacillus lehensis* MLB-2^T (96.013%, Ghosh et al., 2007), *Bacillus oshimensis* K11^T (95.859%, Yumoto et al., 2005) and *Bacillus hunanensis* DSM 23008^T (95.795%, Chen et al., 2011a); the remaining species with validly published names showed less than 94% similarity (see Fig. 3). Considering the low sequence similarity between the novel strain and other bacteria belonging to the genus *Bacillus* and related genera, HNA-14^T may be a representative of a new species.

Conclusions

The chemotaxonomic data, the phenotypic data and the phylogenetic analysis based on 16S rRNA gene sequences suggested that strain HNA-14^T represents a member of the genus *Bacillus*. However, there were still several differences between the strain and its phylogenetic relatives in physiological characteristics (see Table 1 and Fig. 2). In conclusion, a novel species of the genus *Bacillus*, *Bacillus shacheensis* sp. nov., is proposed.

Description of *Bacillus shacheensis* sp. nov.

Bacillus shacheensis (sha.che.en'sis. N.L. masc. adj. *shacheensis* pertaining to Shache County, Xinjiang Province, China, the source of the sample from which the type strain was isolated)

Cells are obligate aerobic, Gram-staining-positive, motile rods (0.5−0.8 × 1.0−1.5 μm). Ellipsoidal spores lie subterminally, and sporangia are unswollen. Colonies are circular, smooth and yellowish. Growth occurs with 0.5−20% (w/v) NaCl (optimum 5−10%), at pH 7.0−10 (optimum pH 8.0) and at 5−40°C (optimum 25−30°C). Hydrolysis of casein, gelatin, starch and Tween 40 is observed, but Tween 20, 60 and 80 are not hydrolyzed. Oxidase and catalase reactions are positive. Nitrate (NO_3) is not reduced to nitrite (NO_2). Negative for urease, egg yolk reaction, methyl red, Voges-Proskauer, H_2S and indole production tests. Positive for Biolog GEN III MicroStation substrates: D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, stachyose, tetrazolium violet, D-raffinose, α-D-lactose, D-salicin, β-methyl-D-glucoside, D-sorbitol, D-arabitol, *myo*-inositol, pectin, D-gluconic acid, D-glucuronic acid, glucuronamide, L-lactic acid, D-malic acid, lithium chloride, Tween 40, α-hydroxy-butyric acid, β-hydroxy-D, L-butyric acid, acetoacetic acid, propionic acid, sodium butyrate, sodium bromate. Negative for the utilization of the following substrates: dextrin, vancomycin, tedextrin, D-melibiose, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, *N*-acetyl-D-galactosamine, *N*-acetyl neuraminic acid, α-D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl glucose, D-fucose, L-fucose, L-rhamnose, inosine, 1% sodium lactate, fusidic acid, D-serine, D-mannitol, glycerol, D-glucose-6-PO_4, D-fructose-6-PO_4, D-aspartic acid, D-serine, troleandomycin, rifamycin SV, minocycline, gelatin, glycyl-L-proline, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-pyroglutamic acid, L-serine, lincomycin, guanidine HCl, niaproof-4, D-galacturonic acid, L-galactonic acid lactone, mucic acid, quinic acid, D-saccharic acid, *p*-hydroxy-phenylacetic acid, methyl pyruvate, D-lactic acid

methyl ester, citric acid, α-keto-glutaric acid, bromo-succinic acid, nalidixic acid, potassium tellurite, γ-amino-butryric acid, α-keto-butyric acid, aztreonam, and formic acid. In API-ZYM assays, esterase(C_4), esterase lipase(C_8), valinearylamidase, naphthol-AS-B1-phosphohydrolase and acid phosphatase are present. But alkaline phosphatase, lipase(C_{14}), leucinearylamidase, cystinearylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are absent. The cell wall contains *meso*-diaminopimelic acid as the diagnostic diamino acid; the predominant polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides and two unknown phospholipids. The major isoprenoidoquinone is MK-7. The major cellular fatty acids are *anteiso*-$C_{15:0}$ (43.16%), *iso*-$C_{15:0}$ (11.89%), *iso*-$C_{16:0}$ (7.65%) and *anteiso*-$C_{17:0}$ (7.45%). The DNA G+C content is 48.6 mol%.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HNA-14^T is HQ620634. The type strain is HNA-14^T (=KCTC 33145=DSM 26902), isolated from a saline-alkali soil sample collected in Shache County, Xinjiang Province, China.

Acknowledgments

This research was supported by the Fundamental Research Funds for the central universities.

References

Bagheri, M., Didari, M., Amoozegar, M. A., Schumann, P., Sánchez-Porro, C. et al. (2012) *Bacillus iranensis* sp. nov., a moderate halophile from a hypersaline lake. *Int. J. Syst. Evol. Microbiol*., **62**, 811−816.

Chen, Y.G., Cui, X.L., Pukall, R., Li, H.M., Yang, Y.L. et al. (2007) *Salinicoccus kunmingensis* sp. nov., a moderately halophilic bacterium isolated from a salt minein Yunnan, south-west China. *Int. J. Syst. Evol. Microbiol*., **57**, 2327−2332.

Chen, Y. G., Hao, D. F., Chen, Q. H., Zhang, Y. Q., Liu, J. B. et al. (2011a) *Bacillus hunanensis* sp. nov., a slightly halophilic bacterium isolated from non-saline forest soil. *Antonie van Leeuwenhoek*, **99**, 481−488.

Chen, Y. G., Zhang, Y. Q., Chen, Q. H., Klenk, H. P., He, J. W. et al. (2011b) *Bacillus xiaoxiensis* sp. nov., a slightly halophilic bacterium isolated from non-saline forest soil. *Int. J. Syst. Evol. Microbiol*., **61**, 2095−2100.

Chen, Y. G., Zhang, Y. Q., He, J. W., Klenk, H. P., Xiao, J. Q. et al. (2011c) *Bacillus hemicentroti* sp. nov., a moderate halophile isolated from a sea urchin. *Int. J. Syst. Evol. Microbiol*., **61**, 2950−2955.

Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S. et al. (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.

Cowan, S. T. and Steel, K. J. (1965) Manual for the Identification of Medical Bacteria, Cambridge University Press, London.

Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **39**, 783−791.

Fritze, D., Flossdorf, J., and Claus, D. (1990) Taxonomy of alkaliphilic bacillus strains. *Int. J. Syst. Evol. Microbiol*., **40**, 92−97.

Ghosh, A., Bhardwaj, M., Satyanarayana, M., Khurana, M., Mayilraj, M. et al. (2007) *Bacillus lehensis* sp. nov., an alkalitolerant bacterium isolated from soil. *Int. J. Syst. Evol. Microbiol*., **57**, 238−242.

Ghosh, A., Paul, D., Prakash, D., Mayilraj, S., and Jain, R. K. (2006) *Rhodococcus imtechensis* sp. nov., a nitrophenol-degrading actinomycete. *Int. J. Syst. Evol Microbiol*., **56**, 1965−1969.

Hong, S. W., Park, J. M., Kim, S. J., and Chung, K. S. (2012) *Bacillus eiseniae* sp. nov., a swarming, moderately halotolerant bacterium isolated from the intestinal tract of an earthworm (*Eisenia fetida* L.). *Int. J. Syst. Evol. Microbiol*., **62**, 2077−2083.

Jukes, T. H. and Cantor, C. R. (1969) Evolution of protein molecules. *In* Mammalian Protein Metabolism, Vol. 3, ed. by Munro, H. N., Academic Press, New York, pp. 21−132.

Kroppenstedt, R. M. (1982) Separation of bacterial menaquinones by HPLC using reverse phase (RP 18) and a silver loaded ion exchanger as stationary phases. *J. Liq. Chromatogr*., **5**, 2359−2387.

Logan, N. A., Popovitch, T. J., and Hoffmaster, A. (2007) *Bacillus* and related genera. *In* Manual of Clinical Microbiology, 9th ed., Vol. 1, ed. by Murray, P. R., Baron, E. J., Jorgensen, J. H., Pfaller, M. A., and Yolken, R. H., American Society for Microbiology, Washington, DC, pp. 445−460.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M. et al. (1984) An integrated procedure for the extraction of is oprenoid quinines and polar lipids. *J. Microbiol. Methods*, **2**, 233−241.

Mesbah, M., Premachandran, U., and 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.

Nielsen, P., Fritze, D., and Fergus, G. (1995) Phenetic diversity of alkaliphilic *Bacillus* strains: Proposal for nine new species. *Microbiology*, **141**, 1745−1761.

Pandey, K. K., Mayilraj, S., and Chakraborti, T. (2002) *Pseudomonas indica* sp. nov., a novel butane-utilizing species. *Int. J. Syst. Evol. Microbiol*., **52**, 1559−1567.

Schleifer, K. H. (1985) Analysis of the chemical composition and primary structure of murein. *Methods Microbiol*., **18**, 123−156.

Schleifer, K. H. and Kandler, O. (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol. Rev*., **36**, 407−477.

Seiler, H., Schmidt, V., Wenning, M., and Scherer S. (2012) *Bacillus kochii* sp. nov., isolated from foods and a pharmaceuticals manufacturing site. *Int. J. Syst. Evol. Microbiol*., **62**, 1092−1097.

Skerman, V. B. D. (1967) A Guide to the Identification of the Genera of Bacteria, 2nd ed., Williams & Wilkins, Baltimore.

Smibert, R. M. and Krieg, N. R. (1994) Phenotypic characterization. *In* Methods for General and Molecular Bacteriology, ed. by Gerhardt, P., Murray, R. G. E., Wood, W.A., and Krieg, N. R., American Society for Microbiology, Washington, DC, pp. 607−654.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. et al. (2011). MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol*., **28**, 2731−2739.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997) The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res*., **25**, 4876−4882.

Zhang, Y. Z., Chen, W. F., Li, M., Sui, X. H., and Liu, H. C. (2012) *Bacillus endoradicis* sp. nov., an endophytic bacterium isolated from soybean root. *Int. J. Syst. Evol. Microbiol*., **62**, 359−363.