The Journal of Microbiology (2012) Vol. 50, No. 2, pp. 349–353
DOI 10.1007/s12275-012-1206-6

NOTE

Phycicoccus ochangensis sp. nov., Isolated from Soil of a Potato Cultivation Field[§]

Hyangmi Kim[1,3], Hyun-Woo Oh[2], Doo-Sang Park[1], Kang Hyun Lee[1], Sung Uk Kim[2], Hee-Moon Park[3], and Kyung Sook Bae[1]*

[1]Microbiological Resource Center, [2]Industrial Bio-materials Research Center, KRIBB, Daejeon 305-806, Republic of Korea
[3]Department of Microbiology & Molecular Biology, Chungnam National University, Daejeon 305-764, Republic of Korea

(Received April 21, 2011 / Accepted December 5, 2011)

Two novel, Gram-positive, motile, coccal bacteria, strains L1b-b9^T and B5a-b5, were isolated from a potato cultivation field in Ochang, Korea. These isolates grew at 10–45°C, pH 5.0–10.0, and in the presence of 8% (w/v) NaCl. The diagnostic diamino acid in the cell-wall peptidoglycan was *meso*-diaminopimelic acid. The major menaquinone was MK-8(H_4) and the main cellular fatty acids were iso-$C_{14:0}$, iso-$C_{15:0}$, and anteiso-$C_{15:0}$. Polar lipids in strain L1b-b9^T consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and an unknown glyco-amino lipid. The G+C content of genomic DNA was 73.6 mol%. A phylogenetic analysis based on 16S rRNA gene sequences showed that strains L1b-b9^T and B5a-b5 shared 99.36% similarity and formed a robust clade with the type species of the genus *Phycicoccus*. Strain L1b-b9^T is related most closely to *Phycicoccus cremeus* V2M29^T (97.52% 16S rRNA gene sequence similarity). On the basis of phylogenetic characteristics, the name *Phycicoccus ochangensis* sp. nov. is proposed for strain LIb-b9^T (=KCTC 19694^T =JCM 17595^T).

Keywords: *Phycicoccus ochangensis*, potato field, *Intrasporangiaceae*

LMOs (Living modified organisms) refer to any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology. Although, there is no clear evidence, concerns have been raised that LMOs may cause alteration of the microbial population in their rhizosphere (Dunfield and Germida, 2001). During the study of differences in the microbial population between an LM-potato cultivation field and a non-LM-potato cultivation field, two novel bacterial strains were isolated: strain L1b-b9^T from the LM-potato cultivation field and strain B5a-b5 from the non-LM-potato cultivation field. The 16S rRNA gene sequence analysis revealed that the strains comprise a distinctive phylogenetic cluster associated with species of the genus *Phycicoccus*, and have a high sequence similarity to each other. At present, we are unable to find any relevance of the strains to the LMOs; however, we report these closely related isolates as an additional species within the genus *Phycicoccus*.

The genus *Phycicoccus* was first proposed by Lee (2006) and at the time of writing comprised six species, *Phycicoccus jejuensis* (Lee, 2006), *Phycicoccus dokdonensis* (Yoon *et al.*, 2008), *Phycicoccus bigeumemsis* (Dastager *et al.*, 2008), *Phycicoccus aerophilus* (Weon *et al.*, 2008), *Phycicoccus cremeus* V2M29^T (Zhang *et al.*, 2011), and *Phycicoccus ginsenosidimutans* BXN5-13^T (Wang *et al.*, 2011). The genus *Phycicoccus* is a member of the family *Intrasporangiaceae*, suborder *Micrococcineae* (Lee, 2006). Members of the genus *Phycicoccus* are Gram-positive, aerobic bacteria with meso-diaminopimelic acid as the diamino acid of the peptidoglycan and MK-8(H_4) as the major menaquinone.

Strains L1b-b9^T and B5a-b5 were isolated from soil samples collected from a potato cultivation field in Ochang by plating serial dilutions onto R2A agar (Difco). The plates were then incubated at 25°C for 3 days. A pure culture was subsequently isolated and stored in a glycerol solution (20%, v/v) at 70°C until use. The stored cells were cultivated aerobically in trypic

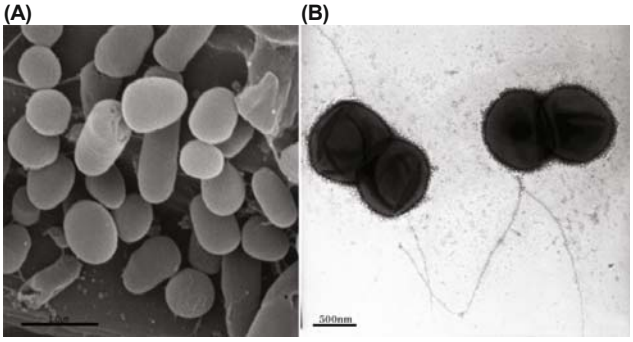

Fig. 1. Scanning electron microscopy of strain L1b-b9^T on R2A agar for 3 days at 25°C (A). Transmission electron micrograph (negative) of strain L1b-b9^T on R2A agar for 3 days at 25°C, showing the presence of flagella (B). Bar, 1 μm (left), 500 nm (right).

*For correspondence. E-mail: ksbae@kribb.re.kr; Tel.: +82-42-860-4610; Fax: +82-42-860-4677
[§]Supplemental material for this article may be found at http://www.springer.com/content/120956

soy broth (TSB) or on tryptic soy agar (TSA) (Difco, USA) for 3 days at 25°C, and then used to determine the physiological characteristics of strains L1b-b9^T and B5a-b5. Media for growth of the isolates were TSA, nutrient agar (NA), and MacConkey agar (all from Difco). All type strains of the four recognized species of the genus *Phycicoccus* were obtained from the KACC (Korean Agricultural Culture Collection), KCTC (Korean Collection for Type Cultures), and NBRC (NITE Biological Resource Center). Gram staining was performed using a Difco Gram stain kit and motility was examined by culturing the isolates in R2A medium that contained 0.5% (w/v) agar. The presence of oxidase activity was determined using an Oxy-swab (bioMérieux, France), and catalase activity was detected by placing drops of 3% (v/v) H_2O_2 on cultures growing on an R2A agar plate, followed by observation of the production of oxygen bubbles. Cells were cultured in pH-adjusted TSB (pH 4.0–11.0 in 0.5 unit increments) at 25°C for 7 days to determine the pH range suitable for growth; in different temperatures of R2A broth ranging from 4 to 55°C for 7 days to determine the optimum

Table 1. Differential characteristics of strains L1b-b9^T, B5a-b5 and the type strains of species of the genus *Phycicoccus*

Strains: 1, L1b-b9^T and B5a-b5; 2, *P. cremeus* V2M29^T; 3, *P. ginsenosidimutans* BXN5-13^T; 4, *P. aerophilus* 5516T-20^T; 5, *P. jejuensis* KSW2-15^T; 6, *P. bigeumensis* MSL-03^T; 7, *P. dokdonensis* DS-8 ^{T}. All data are from this study. +, Positive; w, weakly positive; -, negative. All strains were Gram-positive, catalase-positive, and positive for hydrolysis of esculin, gelatin, and starch, and negative for indole production. In API 20NE and API ID 32GN, all strains assimilate mannose, gluconate, malate, D-saccharose, D-maltose, D-mannitol, and D-glucose, but do not assimilate phenyl-acetate, L-rhamnose, itaconic acid, suberic acid, sodium malonate, sodium acetate, potassium 5-ketogluconate, L-serine, L-arabinose, capric acid, 3-hydroxybutyric acid and L-proline. In API ZYM, all strains are positive for α-glucosidase, but negative for trypsin, naphthol-AS-BI-phosphohydrolase, *N*-acetyl-β-glucosaminidase, and α-fucosidase.

Characteristic	1	2	3	4	5	6	7
Source	Soil	Soil	Soil	Air	Seaweed	Soil	Soil
Cell morphology	Coccus	Rods	Coccus	Short rod	Coccus	Coccus	Coccus
Colony colour	White	Creamy	Grayish yellow	White	Yellow	Yellow	Grayish yellow
Motility	+	+	+	+	+	-	-
Oxidase	-	-	+	-	-	-	+
Nitrate reduction	+	+	+	-	+	+	+
Hydrolysis of DNA	+	-	+	+	+	-	-
Assimilation of:							
Adipate	-	w	-	-	-	-	-
N-Acetyl-glucosamine	-	+	w	-	-	+	+
D-Ribose	-	-	-	-	-	+	+
Inositol	-	-	-	-	-	+	+
Lactic acid	-	-	-	-	-	w	+
L-Alanine	-	+	w	-	-	w	+
Glycogen	+	+	+	+	+	-	-
3-Hydroxybenzoic acid	-	-	-	+	+	-	-
Salicin	-	+	-	+	-	+	+
D-Melibiose	+	+	+	+	+	-	+
L-Fucose	-	-	-	+	-	+	+
D-Sorbitol	-	-	w	-	+	+	+
Propionic acid	-	w	-	-	+	-	-
Valeric acid	-	w	-	-	-	-	-
Trisodium citrate	-	-	-	-	-	+	w
L-Histidine	-	-	-	-	-	+	w
Potassium 2-ketogluconate	-	-	-	-	-	+	w
4-Hydroxybenzoic acid	-	-	-	-	+	-	-
Enzyme activities:	-	-	-	-	-	-	-
Alkaline phosphatase	-	w	w	-	-	-	-
Esterase(C4)	+	w	w	+	-	-	-
Esterase Lipase(C8)	+	-	-	+	+	-	-
Lipase(C14)	-	+	+	-	-	+	+
Leucine arylamidase	+	-	-	+	+	-	-
Valine arylamidase	w	-	-	-	-	-	-
Crystine arylamidase	w	-	-	-	-	-	-
α-Chymotrypsin	-	w	+	w	-	-	+
Acid phospatase	+	w	w	w	+	-	w
α-Galactosidase	-	w	+	-	-	+	+
β-Galactosidase	+	-	-	+	+	-	-
β-Glucuronidase	-	+	+	-	-	+	+
β-Glucosidase	+	-	-	+	+	-	-
α-Mannosidase	w	-	-	-	-	-	-
DNA G+C content (mol%)	73.6	72.0	72.0	71.2	74.2	73.4	70.9

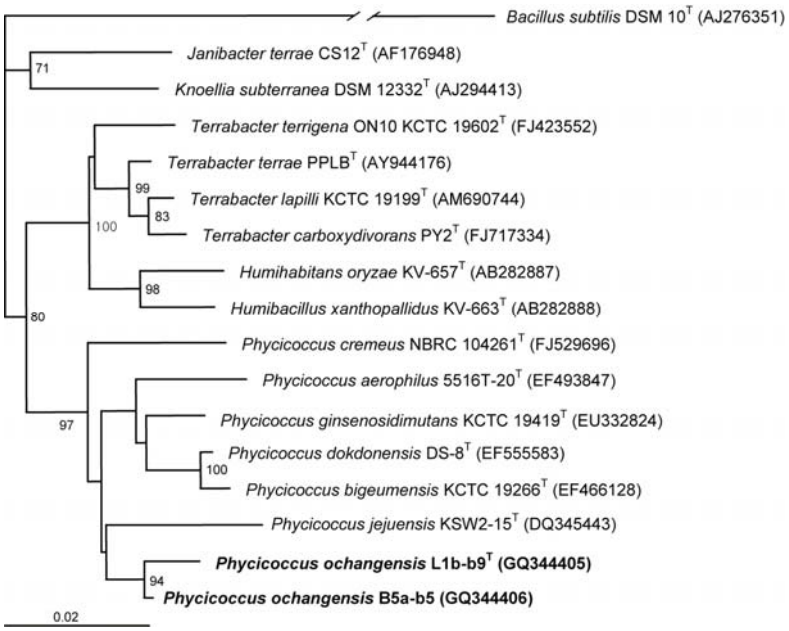

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strains L1b-b9^T, B5a-b5, and related taxa. Bootstrap values (expressed as percentages of 1,000 replications) > 50% are shown at branching points. Bar, 0.02 substitutions per nucleotide position.

growth temperature; and in the presence of NaCl concentrations ranging from 0–10% (w/v) for 7 days to determine tolerance to NaCl in the nutrient broth. Growth was evaluated by measuring the optical density at 595 nm using a microplate reader (Bio-Rad, USA).

The hydrolysis of starch, casein, xylan, pectin, phenylalanine, Tween 20, 40, 60, and 80, CM-cellulose, and DNA was conducted as described by Smibert and Krieg (1994). API 20NE, API ID 32GN, and API ZYM test strips (bioMérieux) were used to analyze the biochemical and physiological traits and sugar assimilation patterns of bacterial strains according to the manufacturer's instructions. GP2 MicroPlates (BIOLOG), containing 95 different carbon compounds, were used to determine substrate oxidation. Anaerobic growth was tested by plating the isolates on an R2A agar plate supplemented with nitrate that was subsequently incubated in a sealed container with a BBL GasPak Pouch (Becton Dickinson, USA). All tests were conducted at 25°C (except API ZYM; 37°C). The morphology and size of the cells were examined using phase-contrast microscopy, a Nikon Optiphot-2 light microscope (Nikon, Japan), a scanning electron microscope (HITACHI S4300N, Japan), and a transmission electron microscope (Philips CM20, Netherlands).

Single cells of strain L1b-b9^T were observed to be cocci (0.5–1.0 μm in diameter) (Fig. 1). Both strains L1b-b9^T and B5a-b5 were Gram-positive, motile (flagella present), nitrate-reducing, and capable of growth under aerobic conditions. The strains were capable of growth on NB containing 0.5–8% NaCl; grew at temperatures from 10 to 45°C, with optimum growth at 20–30°C, and at pH 5.0–10.0, with optimum growth at pH 6.0–8.0. On R2A medium, both strains grew as white, circular, smooth, convex colonies, approximately 1 mm in diameter at one week. Cells grew on TSA, NA, and MacConkey agar. Both isolates hydrolyzed casein, starch, DNA, Tween 40 and Tween 80, but not Tween 20, Tween 60, CM-cellulose, xylan, pectin, or phenylalanine. The morphological, physiological and biochemical characteristics of strains L1b-b9^T and B5a-b5 and the type strains of related species are shown in Table 1.

Analyses of diaminopimelic acid and sugars were carried out by cellulose TLC (Merck) according to Komagata and Suzuki (1987). Isoprenoid quinones were extracted according to the method of Collins and Jones (1981) and then purified using Sep-pak cartridges (silica cartridges; Waters). Menaquinones were analyzed using HPLC (Hitachi L-50000) equipped with a reversed-phase column (YMC pack ODS-A; YMC), as described by Shin *et al.* (1996). Polar lipids were extracted and analyzed by two-dimensional TLC according to Minikin *et al.* (1984). The strains were grown on TSA for 3 days at 25°C to analyze their fatty acid methyl esters, which were extracted and prepared according to standard protocols provided by the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The fatty acids were then analyzed using a gas chromatograph (model 6890N and Auto-sampler 7683; Agilent) and were identified using the Microbial Identification Sherlock software package (classical method, MIDI Sherlock system 4.1, TSBA library version 4.0). Genomic DNA of strain L1b-b9^T was extracted for analysis of G+C content and 16S rRNA gene sequences according to the method described by Sambrook and Russell (2001). The G+C content of genomic DNA was determined using the method described by Tamaoka and Komagata (1984). Briefly, chromosomal DNA was hydrolyzed into nucleosides with nuclease P1 and alkaline phosphatase. The resultant nucleosides were then analyzed by HPLC using a reversed-phase column (Supelcosil LC-18-S; Supelco).

The cell wall of strain L1b-b9^T contained *meso*-diaminopimelic acid as the diamino acid, and glucose and ribose as major cell wall sugars. The predominant menaquinone was MK-8(H_4) and the phospholipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and an unknown glyco-amino lipid (Fig. 3). The predominant fatty acids of strains L1b-b9^T and B5a-b5 were iso-$C_{14:0}$, iso-$C_{15:0}$,

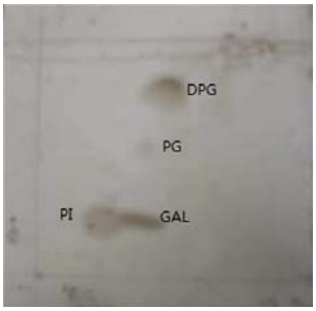

Fig. 3. Two-dimensional TLC of polar lipids from strain L1b-b9^T. Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; GAL, unknown glyco-amino lipid.

and anteiso-$C_{15:0}$ (Table 2). The DNA G+C content of strain LIb-b9^T was 73.6 mol%.

The phylogenetic relationships of strains L1b-b9^T, B5a-b5, and other closely related species were inferred based on their 16S rRNA gene sequences. The 16S rRNA genes of strains L1b-b9^T and B5a-b5 were amplified by PCR using the universal primers 27F and 1492R, as previously described (Kim *et al.*, 2009). The 16S rRNA gene sequences of the isolates were compared with available 16S rRNA gene sequences from GenBank using the BLAST program (http://www.ncbi.nlm.nih.gov/blast/) and EzTaxon server (Chun *et al.*, 2007). The 16S rRNA sequences of the isolates and their relatives were aligned using CLUSTAL X (1.83) (Thompson *et al.*, 1997). Phylogenetic trees were constructed with the PHYLIP program (Felsenstein, 2005) using neighbour-joining (Saitou and Nei, 1987), maximum likelihood (Felsenstein, 1981) and maximum parsimony (Fitch, 1971) methods. The topology of the phylogenetic tree was evaluated with a bootstrap analysis (Felsenstein, 1985) of the neighbour-joining data.

The nearly complete sequences of the 16S rRNA genes of L1b-b9^T and B5a-b5, which were 1,411 bp and 1,343 bp, respectively, were determined and deposited in GenBank under the accession numbers GQ344405 and GQ344406, respectively. The high 16S rRNA gene sequence similarity (99.36%) of the isolated strains to each other suggests that these strains belong to the same species. *P. cremeus* V2M29^T has the highest sequence similarity (97.52%) to the isolated strains and *P. ginsenosidimutans* BXN5-13^T is the next closest species (97.29%); however, the isolates were phylogenetically most closely related to *P. jejuensis* KSW2-15^T in the tree based on neighbour-joining and the maximum parsimony algorithm (Fig. 2 and Supplementary data Fig. S1).

DNA-DNA hybridization was performed using the microplate method described by Ezaki *et al.* (1989). The DNA-DNA hybridization values between the two novel strains exceeded 90%; however, the values of DNA-DNA relatedness between strains LIb-b9^T and the other related type strains were below 50% (Supplementary data Table S1). Consequently, the DNA-DNA hybridization results sup-

Table 2. Cellular fatty acid compositions (%) of some *Phycicoccus* species, strain L1b-b9^T and strain B5a-b5

Strains: 1, L1b-b9^T; 2, B5a-b5; 3, *P. cremeus* V2M29^T; 4, *P. ginsenosidimutans* BXN5-13^T; 5, *P. aerophilus* 5516T-20^T; 6, *P. jejuensis* KSW2-15^T; 7, *P. bigeumensis* MSL-03^T; 8, *P. dokdonensis* DS-8^T. All the data were obtained in this study. -, Not detected; tr, trace amounts (< less than 1.0% of the total).

Fatty acid	1	2	3	4	5	6	7	8
Straight-chain fatty acid								
$C_{15:0}$	7.6	8.1	-	-	5.9	8.2	-	3.0
$C_{16:0}$	tr	tr	tr	tr	2.2	1.3	1.7	tr
$C_{17:0}$	2.1	2.0	2.8	2.1	5.5	7.8	3.0	1.8
Unsaturated fatty acid								
$C_{15:1}$ $\omega6c$	5.5	5.1	4.8	7.7	1.5	1.4	2.3	4.6
$C_{17:1}$ $\omega8c$	4.6	4.8	16.1	11.5	21.2	20.1	3.3	5.6
$C_{17:1}$ $\omega6c$	tr	5.5	-	1.0	-	1.4	tr	1.4
$C_{18:1}$ $\omega9c$	-	-	1.6	1.2	5.8	2.2	-	1.2
Branched fatty acid								
iso-$C_{13:0}$	1.2	2.0	1.3	1.6	tr	-	1.4	1.1
iso-$C_{14:0}$	15.2	20.0	8.3	12.3	4.8	4.8	1.4	8.5
iso-$C_{15:0}$	18.9	29.0	30.9	21.0	18.5	13.3	23.6	39.4
anteiso-$C_{15:0}$	11.8	10.3	4.3	18.0	3.3	6.0	31.0	10.2
iso H-$C_{16:1}$	3.3	2.9	1.5	-	tr	tr	1.0	2.4
iso-$C_{16:0}$	9.2	5.7	12.9	3.4	17.3	9.9	6.9	6.7
iso-$C_{17:0}$	-	-	1.0	-	-	-	tr	-
anteiso-$C_{17:0}$	1.2	1.2	1.5	-	1.5	1.0	3.6	1.1
10-Methyl fatty acid								
$C_{16:0}$	1.2	1.8	-	-	tr	tr	1.1	1.6
$C_{17:0}$	5.4	3.8	2.4	5.9	1.4	11.1	1.7	1.9
Hydroxy fatty acid								
$C_{17:0}$ 3-OH	1.0	1.2	-	-	2.4	3.8	tr	tr
Summed feature*								
1	tr	1.2	1.5	1.1	tr	-	tr	1.6
3	tr	1.4	2.7	2.9	3.3	1.5	2.0	3.7
6	-	-	-	-	tr	1.8	tr	tr

* Summed feature 1 contains iso H-$C_{15:1}$ and/or $C_{13:0}$ 3-OH. Summed feature 3 contains $C_{16:1}$ $\omega7c$ and/or iso $C_{15:0}$ 2-OH. Summed feature 6 contains $C_{19:1}$ $\omega11c$ and/or $C_{19:1}$ $\omega9c$.

ported the proposal of a novel *Phycicoccus* species.

On the basis of the phenotypic and phylogenetic data, strains L1b-b9^T and B5a-b5 should be classified as representatives of a novel species within the genus *Phycicoccus*, for which the name *Phycicoccus ochangensis* sp. nov. is proposed.

Description of *Phycicoccus ochangensis* sp. nov.

Phycicoccus ochangensis (o.chang.en'sis. N.L. masc. adj. ochangensis, pertaining to Ochang)

Cells are Gram-positive, aerobic, motile cocci (0.5–1.0 μm). On R2A medium, colonies are white, round and convex. Growth occurs at 10–45°C (optimum, 20–30°C), at pH 5.0–10.0 (optimum, 6.0–8.0), and in the presence of 0.5–8% (w/v) NaCl. Casein, starch, DNA, Tween 40, and Tween 80 are hydrolyzed, but Tween 20, Tween 60, CM-cellulose, xylan, pectin, and phenylalanine are not. The following compounds are utilized as sole carbon sources: dextrin, glycogen, L-arabinose, D-fructose, D-galactose, α-D-glucose, maltose, maltotriose, D-mannose, D-melezitose, D-melibiose, β-methyl-D galactoside, 3-methyl glucose, palatinose, D-ribose, stachyose, sucrose, turanose, D-xylose, acetic acid, α-ketoglutaric acid, α-ketovaleric acid, L-lactic acid, L-malic acid, propionic acid, pyruvic acid, 2,3-butanediol, glycerol, adenosine, 2'-deoxy adenosine, and thymidine. The cell wall contains *meso*-diaminopimelic acid in the peptidoglycan. The whole-cell sugars are glucose and ribose. The predominant menaquinone is MK-8(H_4). The major cellular fatty acids are iso-$C_{14:0}$, iso-$C_{15:0}$, and anteiso-$C_{15:0}$. Polar lipids include diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and an unknown glyco-amino lipid. The DNA G+C content of the type strain is 73.6 mol%.

The type strain is L1b-b9^T (=KCTC 19694^T =JCM17595^T), isolated from a potato cultivation field in Ochang. B5a-b5 (=KCTC 19695 =JCM 17594) is a second strain of the species.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strain L1b-b9^T and B5a-b5 are GQ344405 and GQ344406, respectively.

This work was supported by a grant from KRIBB research initiative program (KGM0231133) and a National Research Foundation of Korea (NRF) grant funded by the Korean government (MEST, 2007-2001776).

References

Chun, J., Lee, J.H., Jung, Y., Kim, M., Kim, S., Kim, B.K., and 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.

Collins, M.D. and Jones, D. 1981. Distribution of isoprenoid quinine structural types in bacteria and their taxonomic implications. *Microbiol. Rev.* **45**, 316–354.

Dastager, S.G., Lee, J.C., Ju, Y.J., Park, D.J., and Kim, C.J. 2008. *Phycicoccus bigeumensis* sp. nov., a mesophilic actinobacterium isolated from Bigeum Island, Korea. *Int. J. Syst. Evol. Microbiol.* 58, 2425-2428.

Dunfield, K.E. and Germida, J.J. 2001. Diversity of bacterial communities in the rhizosphere and root interior of field-grown genetically modified *Brassica napus*. *FEMS Microb. Ecol.* **38**, 1–9.

Ezaki, T., Hashimoto, Y., and Yabuuchi, E. 1989. Fluorometric deoxyribonucleic acid-deoxyribonucleic acid ybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Bacteriol.* **39**, 224–229.

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. 2005. PHYLIP (Phylogeny Inference Package), version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

Fitch, W.M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst. Zool.* **20**, 406–416.

Kim, B.C., Jeong, W.J., Kim, D.Y., Oh, H.W., Kim, H.M., Park, D.S., Park, H.M., and Bae, K.S. 2009. *Paenibacillus pueri* sp. nov., isolated from Pu´er tea. *Int. J. Syst. Evol. Microbiol.* **59**, 1002–1006.

Komagata, K. and Suzuki, K. 1987. Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol.* **19**, 161–207.

Lee, S.D. 2006. *Phycicoccus jejuensis* gen. nov., sp. nov., an actinomycete isolated from seaweed. *Int. J. Syst. Evol. Microbiol.* **56**, 2369–2373.

Minnikin, D.E., O'Donnell, A.G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A., and Parlett, J.H. 1984. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J. Microbiol. Methods* **2**, 233–241.

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

Sambrook, J. and Russell, D.W. 2001. Molecular Cloning: a Laboratory Manual, 3rd (edn.). Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, N.Y., USA.

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

Shin, Y.K., Lee, J.S., Chun, C.O., Kim, H.J., and Park, Y.H. 1996. Isoprenoid quinone profiles of the *Leclercia adecarboxylata* KCTC 1036^T. *J. Microbiol. Biotechnol.* **6**, 68-69.

Smibert, R.M. and Krieg, N.R. 1994. Phenotypic characterization. Methods for General and Molecular Bacteriology, pp. 607–654. *In* Gerhardt, P., Murray, R.G.E., Wood, W.A., and Krieg, N.R. (eds.). American Society for Microbiology, Washington, D.C., USA.

Tamaoka, J. and Komagata, K. 1984. Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol. Lett.* **25**, 125–128.

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.

Wang, L., An, D.S., Yi, T.H., Lee, S.T., and Im, W.T. 2011. *Phycicoccus ginsenosidimutans* sp. nov., isolated from soil of a ginseng field. *Int. J. Syst. Evol. Microbiol.* **61**, 524–528.

Weon, H.Y., Yoo, S.H., Kim, B.Y., Schumann, P., Kroppenstedt, R.M., Hong, S.K., and Kwon, S.W. 2008. *Phycicoccus aerophilus* sp. nov., isolated from air. *Int. J. Syst. Evol. Microbiol.* **58**, 2389–2392.

Yoon, J.H., Lee, S.Y., Kang, S.J. and Oh, T.K. 2008. *Phycicoccus dokdonensis* sp. nov., isolated from soil. *Int. J. Syst. Evol. Microbiol.* **58**, 597–600.

Zhang, J.A., Liu, X.Y., and Liu, S.J. 2011. *Phycicoccus cremeus* sp. nov., isolated from forest soil, and emended description of the genus *Phycicoccus*. *Int. J. Syst. Evol. Microbiol.* **61**, 71–75.