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TAXONOMIC DESCRIPTION
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Epidermidibacterium keratini gen. nov., sp. nov., a member of the family *Sporichthyaceae*, isolated from keratin epidermis

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Abstract

A novel actinobacterial strain, designated EPI-7^T, was isolated on R2A agar from human skin (keratinocytes) and subjected to a taxonomic study using a polyphasic approach. Strain EPI-7^T showed a Gram-positive reaction, was non-motile, non-spore-forming, and cells had a rod-shape. Colonies were round, convex and pale yellow. Phylogenetic analysis based on 16S rRNA gene sequences showed that the novel isolate formed a cluster with several uncultured bacterial clones and with cultured members of the genera *Modestobacter* and *Sporichthya*. The 16S rRNA gene sequence similarities with respect to the type strains of recognized species from the above genera and other phylogenetic neighbours ranged from 92.6 to 93.4 %. The G+C content of the genomic DNA was 68.9 mol%. The only isoprenoid quinone was MK-9(H4), and the major fatty acids detected were $C_{17:1}\omega8c$, $C_{16:0}$, iso-$C_{15:0}$ and summed feature 3. The major polar lipids were found to be phosphatidylethanolamine, phosphatidylinositol, three unidentified phospholipids, phosphatidylglycerol, phosphatidylcholine, two unidentified amino lipids and three unidentified lipids. The cell-wall peptidoglycan contained *meso*-diaminopimelic acid, glutamic acid and alanine. Whole-cell sugars present included rhamnose, glucose and galactose. The combination of the genotypic and phenotypic data allowed differentiation of strain EPI-7^T from its closest phylogenetic neighbours and provided evidence that strain EPI-7^T represents a novel genus and species in the family *Sporichthyaceae*. The name *Epidermidibacterium keratini* gen. nov., sp. nov. is proposed with the type strain being EPI-7^T (=KCCM 90264^T=JCM 31644^T).

Humans live in symbiosis with a diverse community of micro-organisms, the composition of which has evolved to carry out many specific tasks that benefit the host as well as to survive and thrive in sites that provide these micro-organisms with a suitable nutrient-filled habitat [1]. The skin is an intricate habitat for many bacteria; a sterile milieu prenatally, human skin soon becomes host to resident bacteria after birth. The type and density of bacteria are determined by anatomic location, local humidity, the amount of sebum and sweat production, and the host's hormonal status and age [2]. Bacterial skin microbiota are commensal, symbiotic or parasitic relative to the host; although alterations in host immune status are known to have a significant impact, the type of relationship established is often inherent to the bacteria [3]. The present study aimed to determine the exact taxonomic position of strain EPI-7^T isolated from human epidermal keratinocytes. The 16S rRNA gene sequence data suggested that the isolate was moderately related to *Modestobacter lapidis* MON 3.1^T [4], *Sporichthya polymorpha* DSM 43042^T [5] and *Modestobacter marinus* 42H12-1^T [6], isolated from sandstone, cultivated soil and deep-sea sediment, respectively. However, 16S rRNA gene sequence similarity values between the novel strain and the type strains of the above-mentioned species were less than 94 %, suggesting that strain EPI-7^T represents a new genus and novel species in the family *Sporichthyaceae*. At present, the family *Sporichthyaceae* contains the single genus *Sporichthya* with two species [7].

Strain EPI-7^T was isolated from human epidermal keratinocytes from the Seongnam region, South Korea. The human epidermal keratinocytes sampled had a pH of 6.5 and a total aerobic microbial cell count of 6.4×10^3 c.f.u. ml^{-1}. For isolation, serially diluted human epidermal keratinocyte samples were spread onto the following solid media (all from Difco): nutrient, R2A, ISP 2 and trypticase soy agars. The plates were incubated at 28 °C for 3 days, after which single colonies were formed on R2A plates. One colony was picked and subcultured on modified R2A agar (yeast extract, 0.5 g; proteose peptone no. 3, 0.5 g; casamino acids, 0.5 g; dextrose, 0.5 g; soluble starch, 0.5 g; sodium pyruvate, 0.3 g; dipotassium phosphate, 0.3 g; magnesium sulfate, 0.05 g;

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Abbreviations: DAP, diaminopimelic acid; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; UPL, unidentified phospholipid.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain EPI-7^T is KX755247.
One supplementary table and two supplementary figures are available with the online version of this article.

agar, 15 g; distilled water 1 l; pH 7.2). The purified strain was designated EPI-7^T.

The genomic DNA of strain EPI-7^T was extracted using an InstaGene Matrix (Bio-Rad). PCR amplification (Bio-Rad) of the 16S rRNA gene was carried out using the 27 F-1492R universal primer pair. Identification of phylogenetic neighbours and pairwise 16S rRNA gene sequence similarities were calculated using the EzBioCloud database (http://ezbiocloud.net/) [8]. Sequence data were aligned using the software package BioEdit [9], and phylogenetic analyses were conducted using MEGA software version 5.05 [10]. Phylogenetic trees were reconstructed using the neighbour-joining [11], maximum-likelihood [12] and maximum parsimony [13] algorithms using 1402 nucleotides as the final dataset, with all positions containing gaps and missing data eliminated. Evolutionary distances were computed using the Kimura 2-parameter method [14], and bootstrap analysis (1000 replications) was performed to determine the stability of the branches.

Cell morphology of strain EPI-7^T grown on R2A agar for 5 days at 25 °C was observed at ×1000 with an Olympus microscope (GX71). Motility was investigated using the hanging-drop technique with fresh cells grown on R2A agar [15]. Electron microscopy samples were treated as described by Lee *et al.* [16], with cells dried at the critical point in CO_2 and sputtered with gold in a sputter coater (SC502, Polaron) and observed using a scanning electron microscope (S4300N, Hitachi; installed in the Korea Basic Science Institute). The Gram-reaction was determined using a bioMérieux Gram stain kit according to the manufacturer's instructions.

Strain EPI-7^T, *M. lapidis* DSM 100206^T, *S. polymorpha* DSM 43042^T and *M. marinus* DSM 45201^T were studied in parallel for the following phenotypic characteristics. Growth at 4, 10, 25, 30, 35 and 40 °C and at pH 4.5–9 (at 0.5 pH unit intervals) was assessed after 5 days of incubation in R2A broth. The pH was adjusted using the following buffer systems [17]: sodium acetate/acetic acid (pH <6) and Tris/HCl (pH 6–9).

Tolerance to salinity was tested in R2A broth supplemented with 0–15.0 % (w/v) NaCl at 0.5 % intervals; results were recorded after 5 days of incubation. Growth on nutrient, ISP 2 and trypticase soy agars was also evaluated at 25 °C for 5 days. Catalase activity was determined by assessing bubble production in 3 % (v/v) H_2O_2, while oxidase activity was determined using 1 % (w/v) tetramethyl phenylenediamine. Tests in the commercial systems BioLog GP2, BioLog Gen III, and API 20NE and API ZYM (bioMérieux) were performed in duplicate according to the manufacturers' instructions. The API ZYM tests were read after 4 h of incubation at 25 °C, and the other API tests were read after at least 48 h at 25 °C. Anaerobic growth was tested in serum bottles by adding sodium thioglycollate (1 g l^{-1}) to R2A broth and substituting the upper airspace with nitrogen gas. Tests for the degradation of DNA, casein, starch, Tween 80 [18] and carboxymethylcellulose [19] were performed and evaluated after 7 days of incubation at 25 °C.

To measure the G+C content of the chromosomal DNA, the genomic DNA of strain EPI-7^T and related taxa was extracted and purified, as described by Price *et al.* [20] and enzymically degraded to nucleotides. The DNA G+C content was determined as described by Mesbah *et al.* [21], using reverse-phase HPLC (Young-lin, Korea). Cell mass for menaquinone and polar lipid analyses of strain EPI-7^T was obtained from cells grown on R2A agar for 96 h at 25 °C. The polar lipid profile of *M. lapidis* DSM 100206^T and *S. polymorpha* DSM 43042^T grown under the same conditions was also determined. Polar lipids were extracted using the procedures described by Komagata and Suzuki [22] and identified by two-dimensional TLC followed by spraying with appropriate detection reagents [23]. Menaquinones were analysed as described by Staneck and Roberts [24] using reversed-phase HPLC. Analysis of the peptidoglycan composition [25, 26] was done using freeze-dried cells, from a 1-week-old culture in R2A medium.

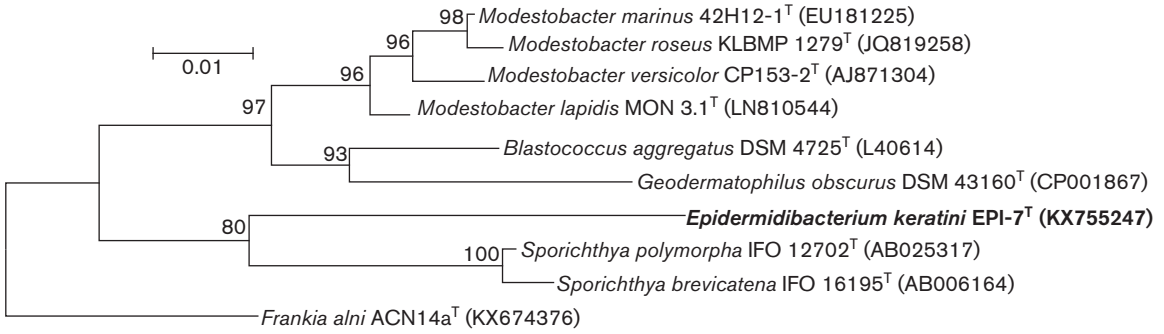

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences of *Epidermidibacterium keratini* gen. nov., sp. nov. EPI-7^T and related taxa. Evolutionary distances were computed using the Kimura 2-parameter method. Bootstrap values greater than 70 % are indicated. Bar, 0.01 substitutions per nucleotide position.

The fatty acid profiles of strain EPI-7^T, *M. lapidis* DSM 100206^T, *M. marinus* DSM 45201^T and *S. polymorpha* DSM 43042^T were determined under identical conditions. The cells were grown on R2A agar for 5 days at 25 °C and collected from the third streak quadrant to obtain cells of the same physiological age. Samples were saponified, methylated and extracted according to the standard protocol of the Sherlock Microbial Identification System [27]. The fatty acids were analysed by gas chromatography (model 6890, Hewlett Packard) and identified using the TSBA40 database of the Microbial Identification software package (MIDI, version 4.5). All experiments were performed in triplicate.

The almost-complete 16S rRNA gene sequence of strain EPI-7^T obtained was a continuous stretch of 1450 bp. Sequence similarity calculations indicated that strain EPI-7^T is a member of the phylum *Actinobacteria* and related to members of the families *Geodermatophilaceae* and *Sporichthyaceae*, with *M. lapidis* MON 3.1^T (93.4 %), *S. polymorpha* DSM 43042^T (93.2 %), *M. marinus* 42H12-1^T (93.0 %), *Modestobacter roseus* KLBMP 1279^T (93.0 %) and *Modestobacter versicolor* CP153-2^T (92.9 %) being the closest relatives. As shown in Fig. 1, the phylogenetic tree based on the neighbour-joining method shows strain EPI-7^T forming a deep and independent branch which then joins the cluster formed by *S. polymorpha* and *Sporichthya brevicatena*. This branch was recovered by all tree-making methods applied and was supported by a bootstrap value of 80 %. The position of strain EPI-7^T suggests that the new isolate is related to the family *Sporichthyaceae* and that it represents a new genus.

The major fatty acids of strain EPI-7^T were $C_{17:1}\omega8c$, C16 : 0, iso-C15 : 0 and summed feature 3 (Table 1). *M. lapidis* DSM 100206^T, *S. polymorpha* DSM 43042^T and *M. marinus* DSM 45201^T grown and analysed under identical conditions showed significant differences from strain EPI-7^T in the respective proportions of $C_{17:1}\omega8c$, $C_{16:0}$, iso-$C_{15:0}$ and summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$), the presence of minor amounts of iso-$C_{16:0}$, $C_{17:0}$, anteiso-$C_{15:0}$, $C_{16:1}\omega9c$, $C_{15:1}\omega8c$ and $C_{18:1}\omega9c$, and the absence of $C_{19:0}$ and $C_{20:0}$ (Table 1). The DNA G+C content of strain EPI-7^T was 68.9 mol%. Strain EPI-7^T contained tetrahydrogenated menaquinone with nine units [MK-9(H4)] as the only isoprenoid quinone; this profile was similar to that of several species of the genus *Modestobacter*, but differed from the profile reported for the two species of the genus *Sporichthya* (Table 2). The polar lipids of strain EPI-7^T were found to be phosphatidylethanolamine (PE), phosphatidylinositol (PI), three unidentified phospholipids (UPLs), phosphatidylglycerol (PG), phosphatidylcholine (PC), two unidentified aminolipids and three unidentified lipids. In turn, *M. lapidis* DSM 100206^T contained PG, PI and several UPLs, but differed in the presence of PC and phosphatidylinositol mannosides (PIM). In the case of *S. polymorpha* DSM 43042^T, PI, an UPL and two unidentified lipids were detected, in addition to diphosphatidylglycerol (DPG) (Fig. S1, available in the online version of this article). The peptidoglycan of strain EPI-7^T was composed of *meso*-diaminopimelic acid (DAP), glutamic acid and alanine and it was assigned to the A1γ type.

Table 1. Cellular fatty acid contents (percentages) of strain EPI-7^T and the type strains of related taxa

Strains: 1, EPI-7^T; 2, *M. lapidis* DSM 100206^T; 3, *S. polymorpha* DSM 43042^T; 4, *M. marinus* DSM 45201^T. Fatty acids that amount to <0.5 % of the total fatty acids in all strains are not shown. –, Not detected. All data from this study.

Fatty acid	1	2	3	4
iso-$C_{14:0}$	1.9	–	–	2.4
$C_{14:0}$	1.6	6.8	1.7	–
iso-$C_{15:0}$	10.1	13.1	–	4.5
anteiso-$C_{15:0}$	3.2	1.6	–	6.5
$C_{15:1}\omega8c$	2.6	–	–	–
$C_{15:1}\omega6c$	1.1	–	–	1.2
iso-$C_{16:1}$ H	1.0		–	9.2
iso-$C_{16:0}$	8.5	4.6	1.7	28.4
$C_{16:1}\omega9c$	3.2	–	–	–
$C_{16:0}$	18.4	14.2	22.4	5.1
iso-$C_{17:0}$				1.6
anteiso-$C_{17:0}$	2.1			6.1
$C_{17:1}\omega8c$	19.9	5.8	21.2	11.8
$C_{17:0}$	7.1	4.4	11.8	4.9
10-Methyl $C_{17:0}$	–	–	8.9	–
$C_{18:3}\omega6c$(6,9,12)		1.4		–
$C_{18:1}\omega9c$	2.5	5.4	10.4	1.1
$C_{18:0}$	1.1	8.7	4.0	
10-Methyl $C_{18:0}$			4.7	
$C_{19:0}$	–	3.6	–	–
$C_{20:0}$	–	6.9	–	–
Summed feature 3	10.0	13.3	9.2	5.4
Summed feature 7	–	1.6	–	
Summed feature 8	1.2	1.9		1.4
Summed feature 9	–		1.8	

Summed feature 3: $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$.

Summed feature 7: $C_{19:1}\omega6c$ and/or $C_{19:1}\omega7c$ and/or cyclo $C_{19:0}$.

Summed feature 8: $C_{18:1}\omega6c$.

Summed feature 9: iso-$C_{17:1}\omega9c$.

The chemotaxonomic results support the recognition of the novel strain as a representative of a novel genus as this profile is different from those of the genera *Sporichthya* and *Modestobacter*, which is also supported by the sequence data. A summary of the chemotaxonomic features of the new isolate and related phylogenetic neighbours is provided in Table 2.

Strain EPI-7^T stained Gram-positive, was aerobic, heterotrophic and consisted of non-motile, non-spore-forming, rod-shaped cells (Fig. S2). Good growth was obtained on R2A agar but not on NA, ISP 2 or TSA. Several physiological/biochemical tests were also useful for differentiating between the novel strain and the species *M. lapidis*,

Table 2. Differential characteristics of strain EPI-7^T and the type strains of phylogenetically related genera

1, EPI-7^T; 2, *S. polymorpha* DSM 43042^T; 3, *S. brevicatena* KCTC 19878^T; 4, *M. lapidis* DSM 100206^T; 5, *M. versicolor* DSM 16678^T; 6, *M. roseus* KCTC 19887^T; 7, *M. marinus* DSM 45201^T. ND, Not determined. All data obtained in this study except where indicated.

Characteristic	1	2	3	4	5	6	7
Cell morphology	Whitish yellow, short rod	Greyish white, short aerial hyphae	Greyish white, short aerial hyphae	Dark orange, short rods and cocci	Pink–deep orange, short rod	Pink, short rod or cocci	Redish orange, short rod
Pigmentation in R2A	−	−	−	−	+	−	+
Growth in 10 % NaCl	+	−	−	−	W	+	−
Major cellular fatty acids (>10 %)*	$C_{17:1}\omega8c$, $C_{16:0}$, iso-$C_{15:0}$ and $C_{16:1}$	$C_{16:0}$, $C_{17:1}\omega8c$, $C_{17:0}$ and $C_{18:1}\omega9c$	iso-$C_{16:0}$	iso-$C_{16:0}$, $C_{15:0}$ and $C_{16:1}$	iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$ and $C_{18:1}$	iso-$C_{16:0}$ and iso-$C_{15:0}$	iso-$C_{16:0}$ and $C_{17:1}\omega8c$
Cell-wall diamino acid	*meso*-DAP	LL-DAP	LL-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP
DNA G+C content (mol%)†	68.9	71.0	71.6	72.0	73.0	71.7	72.3
Whole-cell sugars†	Rhamnose, glucose, galactose	Rhamnose, arabinose, galactose, glucose	Glucosamine, mannose, galactose, glucose	Glucose, galactose, ribose, arabinose	Galactose, ribose, glucose	Galactose, ribose, glucose	Galactose, ribose, glucose
Major quinone	MK-9(H4)	MK-9(H6)	MK-9(H8)	MK-9(H4)	MK-9(H4)	MK-9(H4)	MK-9(H4)
Main polar lipids	PE, PI, PG, PC	DPG, PI	ND	PG, DPG, PE, PI, PIM	PG, DPG, PE, PI	DPG, PE, PI, PIM	PG, DPG, PE, PI

*Fatty acid data for *M. versicolor* and *M. roseus* KLBMP 1279^T obtained from [28] and [29].

†Whole-cell sugars, polar lipids and DNA G+C content (mol%) data for *S. brevicatena* YU720-21^T; *M. lapidis* MON 3.1^T; *M. roseus* KLBMP 1279^T; *M. versicolor* CP153-2^T and *M. marinus* 42H12-1^T obtained from [4, 6, 7, 28] and [29] respectively. Other data from this study.

M. marinus and *S. polymorpha*; these included enzymic activities such as valine arylamidase and α- and β-glucosidase, and carbon source assimilation of maltotriose, 3-methyl glucose, D-galactose and D-mannose. Other physiological characteristics of strain EPI-7^T are summarized in the species description, while comparison of several differential characteristics is given in Table S1.

DESCRIPTION OF *EPIDERMIDIBACTERIUM* GEN. NOV.

Epidermidibacterium (E.pi.der.mi.di.bac.te′ri.um. N.L. n. *epidermis -idis* skin; L. neut. n. *bacterium* a small rod or staff; N.L. neut. *Epidermidibacterium* a small rod from the skin).

Cells are rod-shaped, Gram-stain-positive, oxidase- and catalase-negative, non-motile and non-spore-forming. Aerobic and chemoheterotrophic. MK-9(H4) is the only isoprenoid quinone detected. The main polar lipids are PE, PI, PG and PC. The peptidoglycan is composed of *m*-DAP, glutamic acid and alanine and is of type A1γ. Whole-cell sugars are rhamnose, glucose and galactose. Major fatty acids are $C_{17:1}\omega 8c$, $C_{16:0}$, iso-$C_{15:0}$ and summed feature 3. The DNA G+C content of the type strain of the type species is 68.9 mol%. Phylogenetically related to the family *Sporichthyaceae* in the phylum *Actinobacteria*. The type species is *Epidermidibacterium keratini*.

DESCRIPTION OF *EPIDERMIDIBACTERIUM KERATINI* SP. NOV.

Epidermidibacterium keratini (ke.ra.ti′ni. N.L. gen. neut. n. *keratini* pertaining to keratin).

Possesses the following properties, in addition to those given in the genus description. Cells are 0.7–1.0 µm in length and 0.3–0.5 µm in diameter. Good growth occurs on R2A agar but not on NA, ISP 2 or TSA. At 25 °C, colonies on R2A agar are circular, convex and pale yellow. On R2A agar, growth occurs at 15–35 °C (optimum, 25 °C), but not at 10 or 40 °C. Growth occurs at pH 5.0–8.5 and with NaCl concentrations of up to 10 %; growth is optimal at pH 6.0 in the absence of NaCl. Nitrate is reduced to nitrite. Casein and starch are degraded. DNA and carboxymethylcellulose are not degraded. Results based on the commercial systems BioLog GP2 and BioLog Gen III are given in Table S1. According to the API ZYM gallery, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-chymotrypsin activities are positive; weakly positive activities for alkaline phosphatase and valine arylamidase; lipase (C14), trypsin, α-galactosidase, β-glucuronidase, β-glucosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are negative.

The type strain, EPI-7^T (=KCCM 90264^T=JCM 31644^T), was isolated from human skin (keratinocytes) in Seong-nam City, South Korea. The G+C content of the genomic DNA of the type strain is 68.9 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Reid G, Younes JA, van der Mei HC, Gloor GB, Knight R *et al.* Microbiota restoration: natural and supplemented recovery of human microbial communities. *Nat Rev Microbiol* 2011;9:27–38.
2. Aly R. Cutaneous microbiology. In: Orkin M, Maibach HI, Dahl MV (editors). *Dermatology*. Los Altos: Appleton & Lange; 1991. pp. 22–25.
3. Feingold DS. Bacterial adherence, colonization, and pathogenicity. *Arch Dermatol* 1986;122:161–163.
4. Trujillo ME, Goodfellow M, Busarakam K, Riesco R. *Modestobacter lapidis* sp. nov. and *Modestobacter muralis* sp. nov., isolated from a deteriorated sandstone historic building in Salamanca, Spain. *Antonie van Leeuwenhoek* 2015;108:311–320.
5. Lechevalier MP, Lechevalier HA, Holbert PE. Sporichthya, un nouveau genre de *Streptomycetaceae*. *Ann Inst Pasteur* 1986;114:277–286.
6. Xiao J, Luo Y, Xu J, Xie S, Xu J. *Modestobacter* marinus sp. nov., a psychrotolerant actinobacterium from deep-sea sediment, and emended description of the genus *Modestobacter*. *Int J Syst Evol Microbiol* 2011;61:1710–1714.
7. Tamura T, Hayakawa M, Hatano K. *Sporichthya brevicatena* sp. nov. *Int J Syst Bacteriol* 1999;49:1779–1784.
8. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y *et al.* Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
9. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 1999;41:95–98.
10. Tamura K, Peterson D, Peterson N, Stecher G, Nei M *et al.* MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011;28:2731–2739.
11. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
12. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
13. Kluge AG, Farris JS. Quantitative phyletics and the evolution of anurans. *Syst Zool* 1969;18:1–32.
14. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
15. Doetsch RN. Determinative methods of light microscopy. In: Gerdhardt P, Murray RGE, Costilow RN, Nester EW, Wood WA *et al.* (editors). *Manual of Methods for General Bacteriology*. Washington, DC: American Society for Microbiology; 1981. pp. 21–33.
16. Lee SW, Oh HW, Lee KH, Ahn TY. *Methylobacterium dankookense* sp. nov., isolated from drinking water. *J Microbiol* 2009;47:716–720.
17. Amoozegar MA, Sánchez-Porro C, Rohban R, Hajighasemi M, Ventosa A *et al.* *Bacillus persepolensis* sp. nov., a moderately halophilic bacterium from a hypersaline lake. *Int J Syst Evol Microbiol* 2009;59:2352–2358.
18. Kouker G, Jaeger KE. Specific and sensitive plate assay for bacterial lipases. *Appl Environ Microbiol* 1987;53:211–213.
19. Ten LN, Im WT, Kim MK, Kang MS, Lee ST. Development of a plate technique for screening of polysaccharide-degrading microorganisms by using a mixture of insoluble chromogenic substrates. *J Microbiol Methods* 2004;56:375–382.

20. **Price CW, Fuson GB, Phaff HJ.** Genome comparison in yeast systematics: delimitation of species within the genera *Schwanniomyces*, *Saccharomyces*, *Debaryomyces*, and *Pichia*. *Microbiol Rev* 1978;42:161–193.
21. **Mesbah M, Premachandran U, Whitman WB.** Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* 1989;39: 159–167.
22. **Komagata K, Suzuki K.** Lipid and cell wall analysis in bacterial systematics. *Methods Microbiol* 1987;19:161–206.
23. **Lechevalier MP, Lechevalier H.** Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* 1970;20:435–443.
24. **Staneck JL, Roberts GD.** Simplified approach to identification of aerobic *actinomycetes* by thin-layer chromatography. *Appl Microbiol* 1974;28:226–231.
25. **Busti E, Cavaletti L, Monciardini P, Schumann P, Rohde M *et al.*** *Catenulispora acidiphila* gen. nov., sp. nov., a novel, mycelium-forming *actinomycete*, and proposal of *Catenulisporaceae* fam. nov. *Int J Syst Evol Microbiol* 2006;56:1741–1746.
26. **Lechevalier MP, Lechevalier HA.** The chemotaxonomy of actinomycetes. In: Dietz A, Thayer J (editors). *Actinomycete Taxonomy (Special Publication no. 6)*. Arlington, VA: Society for Industrial Microbiology; 1980. pp. 227–291.
27. **Sasser M.** *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note 101. Newark, DE, USA: Microbial ID, Inc.; 1990.
28. **Reddy GS, Potrafka RM, Garcia-Pichel F.** *Modestobacter versicolor* sp. nov., an actinobacterium from biological soil crusts that produces melanins under oligotrophy, with emended descriptions of the genus *Modestobacter* and *Modestobacter multiseptatus* Mevs *et al.* 2000. *Int J Syst Evol Microbiol* 2007;57:2014–2020.
29. **Qin S, Bian GK, Zhang YJ, Xing K, Cao CL *et al.*** *Modestobacter roseus* sp. nov., an endophytic actinomycete isolated from the coastal halophyte *Salicornia* europaea Linn., and emended description of the genus *Modestobacter*. *Int J Syst Evol Microbiol* 2013;63:2197–2202.