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ORIGINAL PAPER

Nakamurella leprariae sp. nov., isolated from a lichen sample

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Abstract

A novel actinobacterium, YIM 132084^T, was isolated from *Lepraria* sp. lichen collected from Yunnan province, south-west PR China and identified by a polyphasic taxonomic approach. The strain was Gram-stain-positive, aerobic, catalase-positive, oxidase-negative, non-motile and coccus-shaped. Colonies were round, convex, smooth and light orange yellow in color. It grew at 10–40 °C (optimum 28 °C), at pH 6.0–11.0 (optimum pH 7.0) and in the presence of 0–4% NaCl (optimum 0%). Strain YIM 132084^T comprised diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol as the major polar lipids, MK-8(H_4) as the predominant menaquinone, and *anteiso*-$C_{15:0}$, *anteiso*-$C_{17:0}$, *iso*-$C_{15:0}$ and *iso*-$C_{16:0}$ as major fatty acids. Strain YIM 132084^T had *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan, and mannose, ribose, glucose and rhamnose as whole-cell sugars. The 16S rRNA gene sequence showed high level of similarity with *Nakamurella flavida* KCTC 19127^T (97.7%) and *Nakamurella flava* CGMCC 4.7524^T (97.7%). The G + C content of the genomic DNA was 72.4 mol%. Based on draft genome sequences, strain YIM 132084^T showed an average nucleotide identity value of 76.1% and 74.9%, a digital DNA–DNA hybridization value of 20.9% and 20.6% with the reference strains *Nakamurella flavida* and *Nakamurella flava*, respectively. The results of the phenotypic, chemotaxonomic and phylogenetic analyses showed that strain YIM 132084^T represents a novel species of the genus *Nakamurella*, for which the name *Nakamurella leprariae* sp. nov. is proposed. The type strain is YIM 132084^T (= CGMCC 4.7667^T = NBRC 114280^T = KCTC 49367^T).

Keywords *Nakamurella* · *Nakamurella leprariae* sp. nov. · Polyphasic taxonomy · Lichen

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De-Feng An and Shao-Juan Yang equally contributed to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM132084^T is MZ050064 and the genome sequence is JAERWK000000000.

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Abbreviations

ANI	Average nucleotide identity
dDDH	Digital DNA–DNA hybridization
DPG	Diphosphatidylglycerol
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
APL	Aminophospholipid
PGL	Phosphoglycolipids
GL	Glycolipid

Introduction

The genus *Nakamurella* was proposed by Tao in 2004, to replace the illegitimate genus *Microsphaera* (Yoshimi et al. 1996), and, at the same time, the family of *Nakamurellaceae* replaced *Microsphaeraceae* (Tao et al. 2004). *Nakamurella* species are distributed in different natural ecosystems, including activated sludge (Yoshimi et al. 1996), rock (Lee et al. 2008), soil (Yoon et al. 2007), feces (Kim et al. 2017),

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lichen (Jiang et al. 2020), automobile air conditioning system (Chaudhary et al. 2021), plant (Yan et al. 2020) and bark (Tuo et al. 2016). At the time of writing, the genus *Nakamurella* is composed of 11 species with validly published names and 2 species with not validly published names (https://www.bacterio.net/genus/nakamurella), and *Nakamurella multipartita* is the type species of this genus. During an investigation on the diversity of cultivable actinobacteria from lichen samples collected in Yunnan province, southwest PR China, a new actinobacterium strain YIM 132084^T was isolated from *Lepraria* sp.. The strain was identified by a polyphasic approach, which indicated that it represented a new species of the genus *Nakamurella*.

Materials and methods

Isolation and culture of strains

The lichen *Lepraria* sp. sample was collected from Yunnan province (99° 39′ E, 22° 23′ N), south-west PR China. The lichen sample was transferred into a sterile paper bag and air-dried at 28 °C for 1 week, then washed three times with sterile water and homogenized with 18 ml of sterile 0.1% $Na_4P_2O_7$ using a sterile glass homogenizer. Strain YIM 132084^T was isolated using a standard dilution plate method on humic acid-vitamin (HV) agar (Hayakawa and Nonomura 1987). An isolated colony was selected and further purified on YIM 38 medium (Li et al. 2016). Strain YIM 132084^T was stored in tubes of aqueous glycerol (20%, v/v) and then in a - 80 ℃ refrigerator. The reference strain, *Nakamurella flavida* KCTC 19127^T was obtained from the Korean Collection for Type Cultures (KCTC), Republic of Korea. *Nakamurella flava* CGMCC 4.7524^T was obtained from the China General Microbiological Culture Collection Centre (CGMCC).

Phenotypic and biochemical tests

Cultural characteristics of strain YIM 132084^T were observed after 3 days of incubation under aerobic conditions at 28 °C on YIM 38 medium. Morphological characteristics were observed by transmission electron microscopy (JEM-2100; JEOL). Growth in different culture media was performed using YIM 38 medium, tryptic soy agar (TSA, BD Difco), R2A agar (MB cell, Republic of Korea), Luria–Bertani (LB) agar, International Streptomyces Project Medium 2 (ISP 2, BD Difco) and ISP 4 (BD Difco) at 28 °C for 3 days. Growth at different temperatures (4, 10, 15, 20, 25, 28, 30, 35, 37, 40 and 45 °C) was tested on YIM 38 medium. The pH range for growth (pH 4.0–13.0, at intervals of 1.0 pH unit) was tested in YIM 38 medium at 28 °C. NaCl tolerance test for growth was performed using YIM 38 medium supplemented with different concentrations of NaCl (0–10%, w/v, in increments of 1.0%) at 28 °C. Anaerobic growth was tested after incubation on YIM 38 agar at 28 °C for 14 days using a GasPak EZ Anaerobe Pouch System (Becton Dickinson). Cell motility was determined in semisolid medium (Tittsler and Sandholzer 1936). Oxidase activity was determined by using 1% (w/v) tetramethyl-p-phenylenediamine reagent and catalase activity was determined as the production of bubbles after the addition of 3% (v/v) H_2O_2 (Jiang et al. 2019). The Gram reaction of strain YIM 132084^T was examined using a standard Gram reaction and was confirmed by the 3% KOH lysis test (Buck 1982). Hydrolysis of starch, cellulose, tyrosine and casein, Tweens (20, 40, 60 and 80), gelatin liquefaction, H_2S production, coagulation and peptonization of milk were tested using the methods described by Smibert and Krieg (1994). Susceptibility to antibiotics was tested on YIM 38 medium plate using filter paper containing the following antibiotics: ofloxacin (5 μg), vancomycin (30 μg), ciprofloxacin (5 μg), norfloxacin (10 μg), polymyxin B (300 IU), gentamicin (10 μg), ampicillin (10 μg), chloramphenicol (30 μg), ceftriaxone (30 μg), penicillin G (10 IU), neomycin (30 μg), kanamycin (30 μg), streptomycin (50 μg), novobiocin (5 μg), lincomycin (15 μg) and tetracycline (30 μg). Sole carbon and nitrogen source utilization were determined using Biolog GEN III MicroPlate, other biochemical properties and enzyme activities were tested using API 20NE, API 50CH and API ZYM kits (bioMérieux) according to the manufacturer's instructions.

Phylogenetic analysis and 16S rRNA gene sequencing

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were done as described by Li et al. (2007). The purified product was cloned using the pEASY-T1 sample cloning kit to obtain the almost-complete 16S rRNA gene sequence. The sequence obtained was compared with available 16S rRNA gene sequences of validly named species using the EzBioCloud server databases (https://www.ezbiocloud.net/) (Yoon et al. 2017). Phylogenetic trees were constructed with neighbor-joining (Saitou and Nei 1987), maximum-likelihood (Tamura et al. 2011) and maximum parsimony (Fitch 1971) methods using the software package MEGA version 7.0 (Kumar et al. 2016). Kimura's two-parameter model was used to calculate evolutionary distance matrices (Kimura 1980). Bootstrap values were calculated based on 1000 replications (Felsenstein 1985).

Genomic analysis

The draft genome sequence of strain YIM 132084^T and *Nakamurella flavida* KCTC 19127^T were determined using the Illumina NovaSeq PE150 sequencing platform. The

processed reads data were assembled using SOAPdenovo version 2.04 short sequence group assembly software (Li et al. 2008). The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values were determined based on the genome sequences of YIM 132084^T and closely related species of *Nakamurella* using the EzBioCloud server databases and formula 2 Genome-to-Genome Distance Calculator website (http://ggdc.dsmz.de/ggdc.php) (Meier-Kolthoff et al. 2013), respectively. Gene annotations were conducted through the NCBI prokaryotic genome annotation pipeline.

Chemotaxonomic analysis

The strain YIM 132084^T and the reference strains were cultured on YIM 38 agar at 28 °C for 3 days to obtain the amount needed for chemotaxonomic characterization. Polar lipids were extracted and analyzed by the method of Minnikin et al. (1984). Menaquinones were extracted by the method of Collins et al. (1977) and detected by HPLC (Tamaoka et al. 1983). The composition of cellular fatty acids were extracted and analyzed according to the standard protocol of the Microbial Identification System (MIDI) (Sasser 1990; Kämpfer and Kroppenstedt 1996). Cell wall amino acids and whole-cell sugars were extracted, detected and analyzed according to procedures described by Schleifer and Kandler (1972) and Tang et al. (2009).

Results and discussion

Phenotypic and biochemical tests

Cells of strain YIM 132084^T were Gram-stain-positive, aerobic, non-spore forming, non-motile, coccus-shaped and 0.7–0.9 μm in a diameter (Fig. S1). The strain was found to grow on ISP 2, R2A, TSA, LB and YIM 38 agar, No growth occurs on ISP 4 agar. Susceptibility to ofloxacin, vancomycin, ciprofloxacin, gentamicin and chloramphenicol were positive, and susceptibility to ampicillin, ceftriaxone and penicillin G were negative. In the API ZYM tests, alkaline phosphatase, esterase (C_4), esterase lipase (C_8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase activities were positive, but lipase (C_{14}), β-glucuronidase, *N*-acetyl-β-glucosaminidase and α-fucosidase activities were negative. In the API 20NE strips, hydrolysis of L-arginine, urease, esculine and PNPG were positive, but nitrate reduction, hydrolysis of L-tryptophan, assimilation of D-maltose, capric acid, adipate, malic acid, trisodium citrate and phenylacetate were negative. In the API 50CH strips, acid was produced from D-glucose, D-fructose, D-mannose, esculin citrate, D-sucrose, D-mycose and D-lyxose. The detailed physiological and biochemical characteristics of strain YIM 132084^T are shown in the species description and Table 1.

Phylogenetic analysis and 16S rRNA gene sequencing

The almost-complete 16S rRNA gene sequence of strain YIM 132084^T was 1480 bp (GenBank accession number MZ050064). Phylogenetic analyses based on the 16S rRNA gene sequence of strain YIM 132084^T indicated that it should be recognized as a member of the genus *Nakamurella*. Strain YIM 132084^T showed a high level of similarity with *Nakamurella flavida* KCTC 19127^T (97.7%) and *Nakamurella flava* CGMCC 4.7524^T (97.7%). Phylogenetic trees were constructed by the neighbour-joining, maximum-likelihood and maximum parsimony algorithms based on the 16S rRNA gene sequence (Fig. 1, Fig. S2 and Fig. S3). The results of three tree-making algorithms showed that strain YIM 132084^T groups within the genus *Nakamurella*.

Genomic analysis

Based on the draft genome sequencing, strain YIM 132084^T contained 39 contigs, with a total length of 4,472,446 bp and an N50 length of 232,774 bp (GenBank accession number JAERWK000000000). Based on the genomic annotation, the genome of strain YIM 132084^T contains 4,101 genes, included 4,009 protein-coding genes, 3 rRNA genes, 46 tRNA genes, 3 ncRNA genes and 40 pseudogenes. The DNA G + C content of strain YIM 132084^T was determined to be 72.4 mol% based on the draft genome. The ANI values between strain YIM 132084^T and the type strains of *Nakamurella flavida* KCTC 19127^T and *Nakamurella flava* CGMCC 4.7524^T were 76.1 and 74.9%, respectively. The ANI value was lower than the 95.0% cutoff for species demarcation (Richter and Rossello-Mora 2009). The dDDH values between strain YIM 132084^T and the type strains: *Nakamurella flavida* KCTC 19127^T and *Nakamurella flava* CGMCC 4.7524^T were 20.9 and 20.6%, respectively, which were much lower than the threshold value (70%) recommended for distinguishing novel prokaryotic species (Elnar et al. 2020).

Chemotaxonomic analysis

The polar lipids profile of strain YIM 132084^T contained the predominant compounds diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), an unidentified aminophospholipid (APL), an unidentified glycolipid (GL) and two unidentified phosphoglycolipids (PGL1-2) (Fig. S4). The predominant menaquinone was MK-8(H_4) in agreement with the genus *Nakamurella* (Chaudhary et al. 2021), in addition,

Table 1 Differential characteristics between strain YIM 132084^T and closely related species of the genus *Nakamurella*

Characteristic	1	2	3
Isolation source	Lichen	Soil	*Mentha haplocalyx* Briq
Colony color	Light orange yellow	Light yellow	Brilliant orange yellow
Cell size (μm)	0.7–0.9	0.6–1.2[a]	1.0–1.8[b]
Growth at (°C)	10–40	4–35	4–40
pH	6–11	5–9	6–10
NaCl concentration (%, w/v)	0–4	0–3	0–5
Acidification of D-glucose	−	−	+
Hydrolysis of			
Tween 20	+	−	+
Tween 40	−	−	+
Starch	−	+	+
Casein	−	-	+
Gelatin	−	+	+
Assimilation of			
D-Glucose	−	−	+
L-Arabinose	−	−	+
D-Mannose	−	−	+
D-Mannitol	−	−	+
N-acetyl-D-glucosamine	−	−	+
Potassium gluconate	−	−	+
Enzyme activity			
Cystine arylamidase	+	+	−
Trypsin	−	+	−
Chymotrypsin	−	+	−
β-Galactosidase	−	+	+
β-Glucosidase	−	+	+
Acid produced from			
Glycol	−	−	+
Erythritol	−	−	+
L-Arabinose	−	−	+
D-Ribose	−	+	−
L-Xylose	−	+	+
L-Sorbose	−	+	−
N-Acetylglucosamine	−	+	+
D-Cellobiose	−	−	+
D-Maltose	+	−	+
D-Melibiose	−	−	+
D-Trehalose	+	−	+
Inulin	−	+	−
D-Melezitose	−	−	+
D-Raffinose	−	+	+
D-Gentiobiose	−	−	+
D-Turanose	+	−	+
Susceptibility to Antibiotics			
Norfloxacin	+	+	−
Polymyxin B	+	+	−
Neomycin	+	+	−
Kanamycin	+	+	−
Streptomycin	−	+	−
Novobiocin	−	+	−

Table 1 (continued)

Lincomycin	−	+	−
Tetracycline	−	+	−
DNA G+C content (mol%)	72.4	72.4	71.6[b]

Strains: 1, YIM 132084^T; 2, *Nakamurella flavida* KCTC 19127^T; 3, *Nakamurella flava* CGMCC 4.7524^T. +, Positive; −, negative. All data were obtained from this study except where indicated

Milk coagulation and peptonization, H_2S production, hydrolysis of cellulose, tyrosine, Tween 60 and Tween 80 were negative in all strains. In API 20NE tests, all strains were positive for hydrolysis of L-arginine, urease, esculine and PNPG. In the API ZYM kits, all strains were positive for alkaline phosphatase, esterase (C_4), esterase lipase (C_8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase. In the API 50CH kits, all strains were positive for acid production from D-glucose, D-fructose, D-mannose, esculin citrate, D-sucrose and D-lyxose

[a]Data from Yoon et al. (2007)

[b]Data from Yan et al. (2020)

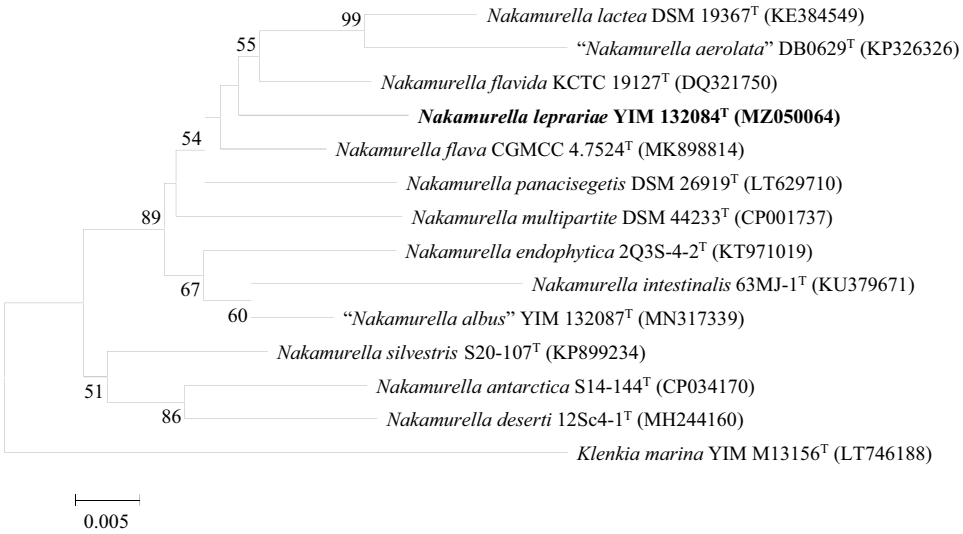

Fig. 1 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of strain YIM 132084^T in relation to its nearest phylogenetic neighbors. Numbers at nodes indicate the level of bootstrap support (> 50%) based on 1000 resamplings. *Klenkia marina* YIM M13156^T (LT746188) was used as an outgroup. Bar, 0.005 substitutions per nucleotide position

MK-8(H_2) and MK-7(H_4) were detected in strain YIM 132084^T. The major cellular fatty acids consist of *anteiso*-$C_{15:0}$ (27.9%), *anteiso*-$C_{17:0}$ (20.7%), *iso*-$C_{15:0}$ (12.5%) and *iso*-$C_{16:0}$ (16.0%), which were similar to other members of the genus *Nakamurella*. The fatty acids composition and content comparison between strain YIM 132084^T and other closely related species of the genus *Nakamurella* are shown in Table 2. Strain YIM 132084^T had *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan, which concurs with the members of the genus *Nakamurella* (Da et al. 2019). The whole-cell sugars detected in strain YIM 132084^T were mannose, ribose, glucose and rhamnose while the whole-cell sugars of *Nakamurella flavida* KCTC 19127^T were galactose, mannose, xylose and rhamnose, and the whole-cell sugars of *Nakamurella flava* CGMCC 4.7524^T were mannose, glucose and rhamnose.

In conclusion, based on phenotypic, chemotaxonomic and phylogenetic analyses, strain YIM 132084^T is considered to represent a novel species of genus *Nakamurella*, for which the name *Nakamurella leprariae* sp. nov. is proposed.

Description of *Nakamurella leprariae* sp. nov.

Nakamurella leprariae (le.pra'ri.ae. N.L. gen. n. *leprariae* referring to the isolation of the organism from the lichen genus *Lepraria*).

Cells are Gram-stain-positive, catalase-positive, oxidase-negative, aerobic, non-motile, non-spore-forming and coccus-shaped (0.7–0.9 μm in diameter). Colonies on YIM 38 medium are round, smooth and convex, light orange yellow in color. Growth occurs at 10–40 °C (optimum 28 °C), at pH 6.0–11.0 (optimum pH 7.0) and at 0–4% NaCl (optimum 0%). The hydrolysis of starch, cellulose, tyrosine and casein, Tweens (40, 60 and 80), gelatin liquefaction, H_2S production, coagulation and peptonization of milk are negative, except the hydrolysis of Tween 20. In the Biolog GEN III system, the following substrates are used as a source of energy: β-methyl-D-glucoside, *N*-acetyl-D-glucosamine,

Table 2 Cellular fatty acid compositions of strain YIM 132084^T and other closely related species of the genus *Nakamurella*

Fatty acid	1	2	3
Straight-chain			
$C_{16:0}$	5.8	**14.5**	6.9
$C_{17:0}$	1.2	**10.5**	1.7
$C_{18:0}$	3.0	3.4	4.3
Branched			
Anteiso-$C_{15:0}$	**27.9**	**37.2**	**21.7**
Anteiso-$C_{16:0}$	0.4	2.1	1.4
Anteiso-$C_{17:0}$	**20.7**	**10.5**	**13.8**
Iso-$C_{14:0}$	0.7	0.5	2.3
Iso-$C_{15:0}$	**12.5**	**13.0**	**12.4**
Iso-$C_{16:0}$	**16.0**	3.8	7.1
Iso-$C_{17:0}$	8.2	1.8	7.2
Summed feature 3[a]	1.5	0.9	5.4

Strains: 1, YIM 132084^T; 2, *Nakamurella flavida* KCTC 19127^T; 3, *Nakamurella flava* CGMCC 4.7524^T. Values are percentages of total fatty acids. The major fatty acids (greater than 10.0%) are shown bold. The data of YIM 132084^T, *Nakamurella flavida* KCTC 19127^T and *Nakamurella flava* CGMCC 4.7524^T were obtained from this study

[a]Summed features represent groups of two fatty acids that could not be separated by HPLC with the Microbial Identification System (MIDI, Inc.). Summed feature 3 consisted of $C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c*

N-acetyl-β-D-mannosamine, *N*-acetyl-D-galactosamine, D-mannose, D-fructose, D-galactose, D-mannitol, D-arabitol, myo-inositol, glycerol, D-glucose-6-phosphate, D-fructose-6-phosphate, D-aspartic acid, L-aspartic acid, L-glutamic acid, L-histidine, L-pyroglutamic acid, L-serine, D-glucuronic acid, D-saccharic acid, L-lactic acid, citric acid, *α*-keto-glutaric acid, D-malic acid, L-malic acid and bromo-succinic acid. The major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol. The predominant menaquinone is MK-8(H_4). The major fatty acids are *anteiso*-$C_{15:0}$, *anteiso*-$C_{17:0}$, *iso*-$C_{15:0}$ and *iso*-$C_{16:0}$. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid as the diagnostic diamino acid, and mannose, ribose, glucose and rhamnose as whole-cell sugars. The G + C content of the genomic DNA is 72.4 mol%.

The type strain, YIM 132084^T (= CGMCC 4.7667^T = NBRC 114280^T = KCTC 49367^T) was isolated from *Lepraria* sp. lichen collected from Yunnan province, south-west PR.

China.

The GenBank accession number for the 16S rRNA gene sequence and draft genome sequence of strain YIM 132084^T are MZ050064 and JAERWK000000000, respectively.

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Author contributions D-FA and S-JY performed the experiments and wrote the manuscript; L-QJ collected the lichen samples; X-YH, X-MC, M-QF, G-DL, and LL analyzed the data; X-YW identified the lichen samples; YJ and M-GJ guided the experiments and revised the manuscript; C-LJ and L-SW designed the study.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

References

Buck JD (1982) Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. Appl Environ Microbiol 44:992–993

Chaudhary DK, Lee H, Dahal RH, Kim DY, Cha IT, Lee KE, Kim DU (2021) *Nakamurella aerolata* sp. nov., isolated from an automobile air conditioning system. Curr Microbiol 78(1):371–377

Collins MD, Pirouz T, Goodfellow M, Minnikin DE (1977) Distribution of menaquinones in actinomycetes and corynebacteria. J Gen Microbiol 100:221–230

Da X, Zhao Y, Zheng R et al (2019) *Nakamurella antarctica* sp. nov., isolated from Antarctica South Shetland Islands soil. Int J Syst Evol Microbiol 69(12):3710–3715

Elnar AG, Kim MG, Lee JE et al (2020) *Acinetobacter pullorum* sp. nov., isolated from chicken meat. J Microbiol Biotechnol 30(4):526–532

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

Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20:406–416

Hayakawa M, Nonomura H (1987) Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. J Ferment Technol 65(5):501–509

Jiang LQ, Zhang K, Li GD et al (2019) *Rubellimicrobium rubrum* sp. nov., a novel bright reddish bacterium isolated from a lichen sample. Antonie Van Leeuwenhoek 112(12):1739–1745

Jiang LQ, An DF, Zhang K et al (2020) *Nakamurella albus* sp. nov.: a novel actinobacterium isolated from a lichen sample. Curr Microbiol 77:1896–1901

Kämpfer P, Kroppenstedt RM (1996) Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. Can J Microbiol 42:989–1005

Kim SJ, Cho H, Joa JH, Hamada M, Ahn JH, Weon HY, Kwon SW (2017) *Nakamurella intestinalis* sp. nov., isolated from the faeces of *Pseudorhynchus japonicus*. Int J Syst Evol Microbiol 67(8):2970–2974

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

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Lee SD, Park SK, Yun YW, Lee DW (2008) *Saxeibacter lacteus* gen. nov., sp. nov., an actinobacterium isolated from rock. Int J Syst Evol Microbiol 58:906–909

Li WJ, Xu P, Schumann P, Zhang YQ, Pukall R et al (2007) *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China). Int J Syst Evol Microbiol 57:1424–1428

Li R, Li Y, Kristiansen K, Wang J (2008) SOAP: short oligonucleotide alignment program. Bioinformatics 24:713–714

Li GD, Chen X, Li QY et al (2016) *Tessaracoccus rhinocerotis* sp. nov., isolated from the faeces of *Rhinoceros unicornis*. Int J Syst Evol Microbiol 66(2):922–927

Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinf 14:60

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

Richter M, Rossello-Mora R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci USA 106:19126–19131

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) Identifcation of bacteria by gas chromatography of cellular fatty acids. Technical Note 101. MIDI, Newark

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, DC, pp 607–654

Tamaoka J, Katayama-Fujimura Y, Kuraishi H (1983) Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. J Appl Bacteriol 54:31–36

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

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

Tao TS, Yue YY, Chen WX, Chen WF et al (2004) Proposal of *Nakamurella* gen. nov. as a substitute for the bacterial genus *Microsphaera* Yoshimi et al. 1996 and *Nakamurellaceae* fam. nov. as a substitute for the illegitimate bacterial family *Microsphaeraceae* Rainey et al. 1997. Int J Syst Evol Microbiol 54:999–1000

Tittsler RP, Sandholzer LA (1936) The use of semi-solid agar for the detection of bacterial motility. J Bacteriol 31:575–580

Tuo L, Li FN, Pan Z, Lou I, Guo M, Ming-Yuen Lee S, Chen L, Hu L, Sun CH (2016) *Nakamurella endophytica* sp. nov., a novel endophytic actinobacterium isolated from the bark of *Kandelia candel*. Int J Syst Evol Microbiol 66(3):1577–1582

Yan XR, Chen MS, Yang C, An MB, Li HY, Shi HC, Tuo L (2020) *Nakamurella flava* sp. nov., a novel endophytic actinobacterium isolated from Mentha haplocalyx Briq. Int J Syst Evol Microbiol 70(2):835–840

Yoon JH, Kang SJ, Jung SY, Oh TK (2007) *Humicoccus flavidus* gen. nov., sp. nov., isolated from soil. Int J Syst Evol Microbiol 57(Pt 1):56–59

Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017) Introducing EzBiocloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67:1613–1617

Yoshimi Y, Hiraishi A, Nakamura K (1996) Isolation and characterization of *Microsphaera multipartita* gen. nov., sp. nov., a polysaccharide accumulating gram-positive bacterium from activated sludge. Int J Syst Bacteriol 46:519–525