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Nakamurella albus sp. nov.: A Novel Actinobacterium Isolated from a Lichen Sample

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Abstract

A novel actinobacterium, YIM 132087^T, isolated from *Lepraria* sp. lichen collected from Yunnan province, south-west PR China. Cells are Gram-stain-positive, catalase-positive and oxidase-negative, aerobic, non-motile and short rod-shaped. Colonies are asporogenous, circular and white brown in colour. Optimal growth occured at 15−35 °C (optimum 28 °C), at pH 5.0−9.0 (optimum pH 6.0), and in the presence of 3% NaCl (w/v). The DNA G+C content of strain YIM 132087^T based on the draft genome sequence was 71.3 mol%. Phylogenetic analysis based on 16S rRNA gene sequences suggested that strain YIM 132087^T belonged to the genus *Nakamurella* and exhibited high levels of 16S rRNA gene sequence similarity with *Nakamurella endophytica* CGMCC 4.7038^T (97.9%) and *Nakamurella intestinalis* NBRC 111844^T (97.2%). The DNA–DNA hybridization values between strain YIM 132087^T and its closest relatives are lower than 26%. Strain YIM 132087^T had meso-diaminopimelic acid as the diagnostic cell-wall diamino acid, and MK-8(H_4) as the predominant menaquinone. Predominant cellular fatty acids (> 10%) were iso-$C_{16:0}$, iso-$C_{15:0}$, $C_{16:0}$ and anteiso-$C_{15:0}$. The polar lipid profile were found to be diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylinositol, three unknown phospholipids, one unknown aminophospholipid and one unknown lipid. Based on phenotypic, phylogenetic and chemotaxonomic analysis, strain YIM 132087^T belongs to the genus *Nakamurella* and represents a novel species of the genus *Nakamurella*, for which the name *Nakamurella albus* sp. nov., with type strain YIM 132087^T (=CGMCC 4.7629^T =NBRC 114017^T), is proposed.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 132087^T is MN317339 and the genome sequence is WLYK00000000.

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Introduction

The genus *Nakamurella*, the sole member and type genus of the family *Nakamurellaceae*, was proposed as a substitute for *Microsphaera* by Yoshimi and Tao et al. [1, 2]. At the time of writing, the genus comprises seven species with validly published names (https://www.bacterio.net/nakamurella.html). The members of the genus *Nakamurella* have been isolated from different environments, including activated sludge, rock, plant bark, faeces of insect and soil [1–8].

During a study of the diversity of actinobacteria present in lichen samples, a novel actinobacterium YIM 132087^T was isolated from *Lepraria* sp. lichen collected from Yunnan province, south-west PR China. The aim of this study was to determine the taxonomic status of the novel isolate using a polyphasic approach.

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Materials and Methods

Isolation, Maintenance and Cultural Conditions

A lichen sample was collected from Yunnan province (99° 39′ E, 22° 23′ N), south-west PR China, then immediately transferred to sterile paper bag and air-dried at 28 °C for 7 days. Strain YIM 132087^T was isolated using a standard dilution plate method on humic acid-vitamin agar media [9]. The isolation procedure was performed as described by Liu et al. [10]. The purified strain was maintained on ISP 2 agar slants at 4 °C and as 20% (v/v) glycerol suspensions at − 80 °C, respectively.

Phylogenetic Analysis

Extraction of genomic DNA and amplification of 16S rRNA gene fragments were performed as described by Li et al. [11]. The 16S rRNA gene sequence obtained in this study was compared with sequences from EzBioCloud using blast (https://www.ezbiocloud.net/) [12]. Phylogenetic trees were constructed with the neighbour-joining [13], maximum-likelihood [14] and maximum-parsimony [15], methods using the software package MEGA version 7.0 [16]. Bootstrap analysis with 1000 replications was conducted to estimate the topology of the phylogenetic tree [17].

Phenotypic Characteristics

Cell morphology was examined by phase-contrast microscopy (ECLIPSE Ni-U; Nikon) and transmission electron microscope (JEM-2100, JEOL) after cells grown on ISP 2 medium at 28 °C for 3 days. Most physiological and biochemical tests—including hydrolysis of starch and cellulose, Tweens (20, 40, 60 and 80), coagulation and peptonisation of milk, H_2S production and nitrate reduction—were observed using the methods described by Smibert and Krieg [18]. Gram staining, catalase and oxidase activities as well as motility were investigated using standard methods [19–21]. Anaerobic growth was determined after incubation on ISP 2 for 14 days at 28 °C using the GasPak EZ Anaerobe Pouch System (BD). Different temperatures (4, 8, 10–45 °C at intervals of 5 °C and 37 °C), pH range (4.0–12.0 at intervals of 1.0 pH unit) for strain growth were examined on ISP 2 medium using the buffer system from Xu et al. [22]. Tolerance to NaCl for growth was examined by cultivation in ISP2 agar containing 0−8% NaCl (w/v, at 1% intervals). Other biochemical properties and enzyme activities were further tested using the API 20NE and API ZYM kits according to the manufacturer's instructions. Carbon source utilization patterns were determined using Biolog GEN III MicroPlates (BioMérieux). For phenotypic comparisons, the reference strains *Nakamurella endophytica* CGMCC 4.7038^T and *Nakamurella intestinalis* NBRC 111844^T were grown and tested under identical conditions.

Chemotaxonomic Characterisation

Cell mass of YIM 132087^T and reference strains for chemical analysis were obtained from cultures grown on ISP 2 agar at 28 °C for 3 days. Polar lipids were extracted and analysed by two-dimensional TLC according to the method of Denner et al. [23]. Fatty acids were extracted with the method of Kuykendall et al. [24] and analysed using the standard protocol of the MIDI System (Sherlock version 6.1; database TSBA6) [25]. The menaquinone was investigated by HPLC according to the method described by Tindall [26]. Cell wall amino acids were extracted, detected and analysed according to procedures described by Schleifer and Kandler [27] and Tang et al. [28]. DNA–DNA relatedness experiment was performed between strain YIM 132087^T and its phylogenetically closely related strains *N. endophytica* CGMCC 4.7038^T and *N. intestinalis* NBRC 111844^T using the photobiotin-labelled DNA probes and micro-well method [29, 30].

Genomic Analysis

The whole-genome sequencing of strain YIM 132087^T was performed using paired-end sequencing method with Hiseq 4000 platform. The draft genome was assembled using SOAP denovo version 2.04 [31] and the short oligonucleotide of assembled results was further polished using SOAP aligner 2.21 [32]. Genes annotation were conducted through the NCBI prokaryotic genome annotation pipeline.

Results and Discussion

Phylogenetic and Genomic Analysis

The nearly full-length 16S rRNA gene sequence of strain YIM 132087^T (1490 bp; GenBank accession number MN317339) was obtained. On the basis of 16S rRNA gene sequence comparisons, strain YIM 132087^T is closely related to members of the genus *Nakamurella*, namely *N. endophytica* CGMCC 4.7038^T (97.9%) and *N. intestinalis* NBRC 111844^T (97.2%), less than 97.0% with other currently described type strains of *Nakamurella*. Moreover, phylogenetic trees showed that strain YIM 132087^T formed a distinct lineage into the genus *Nakamurella*, with *Nakamurella intestinalis* NBRC 111844^T as the closely related neighbour in Fig. 1. Similar topologies were also observed in the maximum-likelihood tree (Fig. S1) and maximum-parsimony tree (Fig. S2). The DNA–DNA hybridization

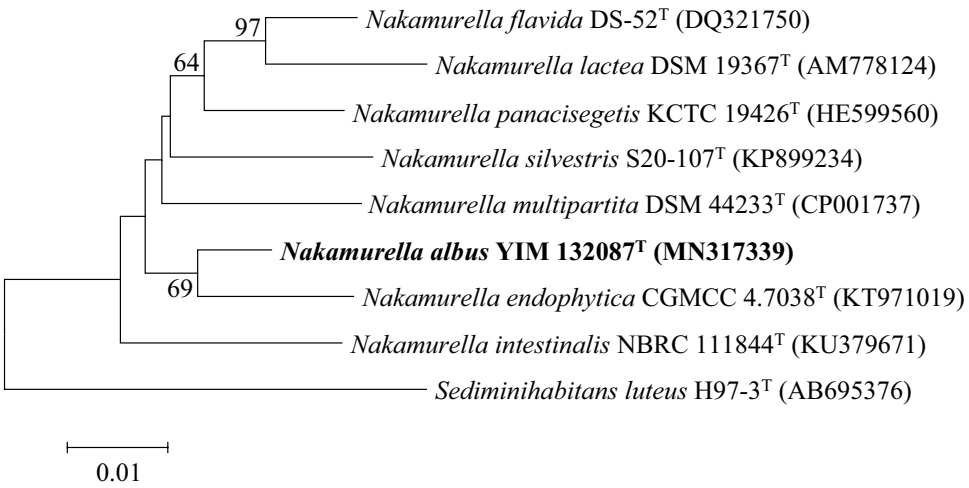

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of strain YIM 132087^T in relation to its nearest phylogenetical neighbours. Numbers at nodes indicate the level of bootstrap support (>50%) based on 1000 resamplings. *Sediminihabitans luteus* H97-3^T (AB695376) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position

values of strain YIM 132087^T with *N. endophytica* CGMCC 4.7038^T and *N. intestinalis* NBRC 111844 ^{T} were 22.1% and 25.3%, respectively, which are lower than the species-level threshold (70%).

The draft genome of strain YIM 132087^T contained 29 contigs, with a total length of 6,051,944 bp and an N50 length of 522,866 bp (GenBank accession number WLYK00000000). Strain YIM 132087^T genome was annotated with 5349 genes, including 5203 protein-coding genes, 4 rRNA genes, 45 tRNA genes, 3 ncRNA genes and 94 pseudogenes. The DNA G+C content of strain YIM 132087^T was determined from the genome to be 71.3 mol%, a value in the range reported for species in the genus *Nakamurella* [8].

Phenotypic Characteristics

After three days of growth (28 °C, ISP 2), strain YIM 132087^T was aerobic, Gram-stain-positive, asporogenous, non-motile and short rod-shaped (0.5−0.7 × 1.0−1.7 μm) (Fig. S3). Colonies of strain YIM 132087^T were white, moist, smooth and round. Strain YIM 132087^T is catalase-positive and oxidase-negative. Showed good growth at 28 °C, at pH 6.0 and in the presence of 1% NaCl. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase and α-mannosidase were positive, but others in the API ZYM system (bioMe´rieux) were found to be negative. In the API 20NE strips, hydrolysis of L-arginine, urease, esculine, gelatin and PNPG, assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate and acide malique were positive, but other tests were negative. Detailed physiological and biological characteristics are shown in novel species description and Table 1.

Chemotaxonomic Characterisation

The polar lipids of strain YIM 132087^T were found to be diphosphatidylglycerol (DPG), phosphatidylmethylethanolamine (PME), phosphatidylethanolamine (PE), phosphatidylinositol (PI), three unknown phospholipids (PL), one unknown aminophospholipid (APL) and lipid (L) (Fig. S4), which was consistent with the genus *Nakamurella*. But the presence of phosphatidylmethylethanolamine and lipid differentiated it from *N. endophytica* CGMCC 4.7038^T, and phosphatidylmethylethanolamine and phospholipid distinguished it from *N. intestinalis* NBRC 111844^T. Predominant cellular fatty acids (> 10%) were identified as iso-$C_{16:0}$ (33.7%), iso-$C_{15:0}$ (12.8%), $C_{16:0}$ (12.6%) and anteiso-$C_{15:0}$ (11.7%). The fatty acid composition was found to be similar to that of the closely related strains *N. endophytica* CGMCC 4.7038^T and *N. intestinalis* NBRC 111844^T, but the proportions of some fatty acids were different (Table 2). The predominant menaquinone was found to be MK-8(H_4) (60.1%), which was uniform with the genus *Nakamurella*. MK-8(H_2) and MK-8 were also found to be considerable amount in strain YIM 132087^T, accounting for 27.3% and 12.6%, respectively. The peptidoglycan of strain YIM 132087^T contained alanine (Ala), glutamic acid (Glu) and meso-diaminopimelic acid as cell-wall amino acids.

Taxonomic Conclusion

In summary, based on the results obtained from phenotypic and biochemical properties as well as from chemotaxonomic and phylogenetic analysis, we conclude that strain YIM 132087^T should be assigned to the genus *Nakamurella* as a novel species, for which the name *Nakamurella albus* sp. nov. is proposed.

Table 1 Phenotypic characteristics that differentiate strain YIM 132087^T from closely related reference strains of the genus *Nakamurella*

Characteristic	1	2	3
Isolation source	Lichen	Plant bark	Faeces
Colony colour	White	Orange-yellow	White
Cell shape	Short rod	Cocci[a], *	Short rod[b], *
Growth at (°C)	15–35	15–40	4–35
pH range for growth	5.0–9.0	5.0–9.0	6.0–9.0
Tolerance of NaCl (%)	0–3	0–2	0–4
Nitrate reduction	−	−	w
Hydrolysis of			
Tween (20, 40, 60)	+	−	−
Gelatin	+	−	+
Starch	+	−	−
Urease	+	+	−
Enzyme activity of (API ZYM)			
α-chymotrypsin	−	+	−
β-galactosidase	−	+	−
α-mannosidase	+	−	−
Assimilation of (API 20NE)			
D-Glucose	+	−	+
L-Arabinose	+	−	+
D-Mannose	+	−	+
D-Manniiol	+	−	+
N-Acetylglucosamine	−	−	+
D-Maltose	+	−	+
Potassium gluconate	+	−	+
Acide caprique	−	−	+
Acide malique	+	−	+
Trisodium citrate	−	−	+
Acide phenylacetique	−	−	+
Fermentation of glucose	−	+	+
Menaquinones (MK)	MK-8(H_4), MK-8(H_2), MK-8	MK-8(H_4)	MK-8(H_4)
Polar lipids	(DPG, PME, PE, APL, PI, PL_{1-3}, L)	(DPG, PE, APL, PI, PL)[a], *	(DPG, PE, APL, PI, L_{1-2})[b], *
Major fatty acids (10%)	iso-$C_{16:0}$, iso-$C_{15:0}$, $C_{16:0}$, anteiso-$C_{15:0}$	iso-$C_{16:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, $C_{16:0}$	$C_{16:0}$, anteiso-$C_{15:0}$, iso-$C_{15:0}$
G+C content mol%	71.3	67.8[a], *	64.6[b], *

Strains: 1, YIM 132087^T; 2, *N. endophytica* CGMCC 4.7038^T; 3, *N. intestinalis* NBRC 111844^T. All data were obtained from this study except where indicated. All strains were negative for hydrolysis of cellulose and Tween 80. In API 20NE tests, all strains were positive for hydrolysis of esculine and PNPG. In the API ZYM kits, all strains were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *α*-glucosidase and *β*-glucosidase

+, positive; −, negative, w, weak

[a], *Data from Tuo et al. [6]

[b], *Data from Kim et al. [8]

Description of *Nakamurella albus* sp. nov.

Nakamurella albus (al'bus. L. masc. adj. *albus* White)

Strain YIM 132087^T is Gram-stain-positive, aerobic, catalase-positive and oxidase-negative, asporogenous, non-motile and short rod-shaped (0.5−0.7 × 1.0−1.7 μm). Colonies on ISP 2 medium are white, moist and circular. Grown well on ISP 2 and YIM 38 medium, weakly on R2A, TSA and NA medium, but not at all on LB agar. Growth occurs at 15−35 °C (optimum 28 °C), at pH 5.0−9.0 (optimum pH 6.0) and in the presence of 3% NaCl. Hydrolysis of Tweens (20, 40, 60), gelatin and starch are positive, but nitrate reduction, H_2S production, milk coagulation and peptonisation, hydrolysis of cellulose are negative. In the Biolog GEN III system, the following

Table 2 Cellular fatty acid profile of strain YIM 132087 ^{T} and closely related reference strains

Fatty acid	1	2	3
Saturated			
$C_{16:0}$	**12.6**	**10.0**	**28.7**
$C_{17:0}$	3.1	6.0	4.2
$C_{18:0}$	1.1	4.6	–
Branched saturated			
Anteiso-$C_{15:0}$	**11.7**	**21.7**	**22.0**
Anteiso-$C_{17:0}$	1.4	**14.9**	3.6
iso-$C_{14:0}$	9.2	1.3	3.2
iso-$C_{15:0}$	**12.8**	8.3	**18.2**
iso-$C_{16:0}$	**33.7**	**22.0**	8.2
iso-$C_{17:0}$	1.7	6.8	1.6
Summed feature 3*	7.0	2.1	9.3

Strains: 1, YIM 132087^{T}; 2, *N. endophytica* CGMCC 4.7038^{T}; 3, *N. intestinalis* NBRC 111844^{T}. All data were obtained from this study. Values are % of total fatty acids. The major fatty acids (greater than 10%) are shown bold. –, Not detected

*Summed feature 3 contained $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$ that cannot be separated by the MIDI system

substrates are used as a source of energy: dextrin, D-maltose, D-trehalose, D-cellobiose, gentiobiose, D-fructose, D-arabitol, inositol, L-histidine, D-galacturonic acid, D-gluconic acid, mucic acid, D-malic acid, γ-amino-butyric acid, α-hydroxy-butyric acid, β-hydroxy-*d*,L-butyric acid, α-keto-butyric acid, acetoacetic acid and acetic acid. The cell-wall peptidoglycan contains meso-diaminopimelic acid as diamino acid. The polar lipid profile comprises diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylinositol, three unknown phospholipids, one unknown aminophospholipid and lipid. The major cellular fatty acids (>10%) are iso-$C_{16:0}$, iso-$C_{15:0}$, $C_{16:0}$ and anteiso-$C_{15:0}$. The predominant menaquinone is MK-8(H_4).

The type strain, YIM 132087^{T} (=CGMCC 4.7629^{T} =NBRC 114017^{T}) was isolated from *Lepraria* sp. lichen collected from Yunnan province, south-west PR China. The DNA G+C content based on the draft genome sequence is 71.3 mol%. The GenBank accession number for the 16S rRNA gene sequence and draft genome sequence of strain YIM 132087^{T} are MN317339 and WLYK00000000, respectively.

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Compliance with Ethical Standards

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

References

1. 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
2. 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
3. Yoon JH, Kang SJ, Jung SY, Oh TK (2007) *Humicoccus flavidus* gen. nov., sp. nov., isolated from soil. Int J Syst Evol Microbiol 57:56–59
4. Kim KK, Lee KC, Lee JS (2012) *Nakamurella panacisegetis* sp. nov. and proposal for reclassification of *Humicoccus flavidus* Yoon et al., 2007 and *Saxeibacter lacteus* Lee et al., 2008 as *Nakamurella flavida* comb. nov. and *Nakamurella lactea* comb. nov. Syst Appl Microbiol 35:291–296
5. 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
6. Tuo L, Li FN, Pan Z, Lou I, Guo M et al (2016) *Nakamurella endophytica* sp. nov., a novel endophytic actinobacterium isolated from the bark of *Kandelia candel*. Int J Syst Evol Microbiol 66:1577–1582
7. França L, Albuquerque L, Zhang DC, Nouioui I, Klenk HP et al (2016) *Nakamurella silvestris* sp. nov., an actinobacterium isolated from alpine forest soil. Int J Syst Evol Microbiol 66:5460–5464
8. Kim SJ, Cho H, Joa JH, Hamada M, Ahn JH et al (2017) *Nakamurella intestinalis* sp. nov., isolated from the faeces of *Pseudorhynchus japonicus*. Int J Syst Evol Microbiol 67:2970–2974
9. Hayakawa M, Nonomura H (1987) Humic acid-vitamin agar, a new medium for the selective isolation of soil acinomycetes. J Ferment Technol 65:501–509
10. Liu CB, Jiang Y, Wang XY, Chen DB, Chen X, Wang LS, Han L, Huang XS, Jiang CL (2017) Diversity, antimicrobial activity, and biosynthetic potential of cultivable actinomycetes with lichen symbiosis. Microb Ecol 74:570–584
11. Li WJ, Xu P, Schumann P, Zhang YQ, Pukall R, Xu LH, Stackebrandt E, Jiang CL (2007) *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China), and emended description of the genus *Georgenia*. Int J Syst Evol Microbiol 57:1424–1428
12. 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
13. Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstucting phylogenetic trees. Mol Biol Evol 4:406–425
14. 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
15. Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20:406–416
16. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874
17. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrat. Evolution 39:783–791
18. 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
19. Leifson E (1960) Atlas of bacterial flagellation. Academic Press, New York
20. Kovacs N (1956) Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature 178:703–703
21. Dong XZ, Cai MY (2001) Determination of biochemical properties. In: Dong XZ, Cai MY (eds) Manual for the systematic identification of general bacteria. Science Press, Beijing (in Chinese), pp 370–398
22. Xu P, Li WJ, Tang SK, Zhang YQ, Chen GZ, Chen HH, Xu LH, Jiang CL (2005) *Naxibacter alkalitolerans* gen. nov. sp. nov., a novel member of the family '*oxalobacteraceae*' isolated from china. Int J Syst Evol Microbiol 55:1149–1153
23. Denner EBM, Paukner S, Kämpfer P, Moore ERB, Abraham WR, Busse HJ, Wanner G, Lubitz W (2001) *Sphingomonas pituitosa* sp. nov., an exopolysaccharide-producing bacterium that secretes an unusual type of sphingan. Int J Syst Evol Microbiol 51:827–841
24. Kuykendall LD, Roy MA, O'Neill JJ, Devine TE (1988) Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. Int J Syst Bacteriol 38:358–361
25. Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101. Newark, DE: MIDI
26. Tindall BJ (1990) Lipid composition of *Halobacterium lacusprofundi*. FEMS Microbiol Lett 66:199–202
27. Schleifer KH, Kandler O (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol Rev 36:407–477
28. 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
29. Ezaki T, Hashimoto Y, Yabuuchi E (1989) Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution 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
30. Christensen H, Angen O, Mutters R, Olsen JE, Bisgaard M (2000) DNA–DNA hybridization determined in microwells using covalent attachment of DNA. Int J Syst Evol Microbiol 50:1095–1102
31. Li R, Li Y, Kristiansen K, Wang J (2008) SOAP: short oligonucleotide alignment program. Bioinformatics 24:713–714
32. Li D, Liu CM, Luo R, Sadakane K, Lam TW (2015) MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics 31:1674–1676