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ORIGINAL ARTICLE

Streptomyces lushanensis sp. nov., a novel actinomycete with anti-cyanobacterial activity

Bing-Huo Zhang[1,2,6], Juan Cheng[1,2,6], Wei Chen[3], Han-Quan Li[2], Jian-Yuan Yang[2], Dong-Jin Park[4], Chang-Jin Kim[4], Rui Shen[1], Yan-Qin Duan[3] and Wen-Jun Li[1,5]

Strain JXJ 0135^T, an anti-cyanobacterial actinomycete, was isolated from a soil sample collected from Lushan Mountain, south China, and identified by using polyphasic approach. Phylogenetic analysis of the near-complete 16S rRNA gene sequence indicated that strain JXJ 0135^T belongs to the genus *Streptomyces* and exhibited distinct subclade and also highest similarity (98.6%) to *Streptomyces scopuliridis* RB72^T. The strain developed well-branched substrate and aerial mycelia, and produced spiral spore chains. Spores were elliptical and the spore surface was smooth. The strain contained *LL*-diaminopimelic acid with whole-cell sugars of mannose, rhamnose, glucose and galactose. Phospholipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol mannosides, phosphotidylinositol dimannoside, an unidentified amino-phospholipid and an unknown phospholipid. The menaquinones were MK-9(H_6) and MK-9(H_8). The major components of the fatty acids were anteiso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$, $C_{16:0}$, iso-$C_{17:0}$ and iso-$C_{14:0}$. The G + C content was 69.3 mol%. The DNA–DNA hybridization value between JXJ 0135^T and *S. scopuliridis* RB72^T was 41.2 ± 1.4%. On the basis of the polyphasic data, strain JXJ 0135^T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces lushanensis* sp. nov. is proposed. The type strain is JXJ 0135^T (= DSM 42121^T = JCM 19628^T = KCTC 29261^T = KACC 17834^T = NRRL B-24994^T).

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INTRODUCTION

Since the first report of livestock poisoning caused by cyanobacteria in 1878,[1] incident of human and animal intoxication by cyanobacterial blooms has occurred with increasing frequency, and the case has become more serious since 1960.[2] Many methods have been developed to manage cyanobacterial blooms;[3–5] however, it is difficult to regulate the occurrence of cyanobacteria by the conventional methods such as biomanipulation and algicides.[6] Several studies have shown that actinomycetes and their metabolites have great potential for controlling cyanobacterial blooms.[7–13]

Actinomycetes are the predominant producer of bioactive compounds, and about 45% of the 22 500 bioactive compounds from microorganisms are produced by actinomycetes.[14] *Streptomyces*, the largest antibiotic-producing genus,[15] is the dominant group of actinomycetes, and 75% of bioactive compounds from actinomycetes are produced by *Streptomyces*.[14] During a search for anti-cyanobacterial microbes, strain JXJ 0135^T exhibiting strong anti-cyanobacterial activity was screened out. The main objective of this study was to determine the taxonomic position of strain JXJ 0135^T.

MATERIALS AND METHODS

Isolation and maintenance of strain

Strain JXJ 0135^T was isolated from a soil sample collected from Lushan Mountain (29°42′ N, 116°26′ E), south China, by using serial dilution technique. The purified strain was incubated on YIM 38# medium[16] at 28 °C and stored as glycerol suspensions (20%, v/v) at −80 °C.

Morphological, cultural, physiological and biochemical characteristics

Cultural characteristics were determined after 2-week incubation at 28 °C on International *Streptomyces* Project (ISP) media.[17] Czapek's agar, potato glucose agar and nutrient agar were prepared according to the method of Dong and Cai.[18] Color determination was carried out by using color chips from the ISCC–NBS color charts (standard samples, no. 2106).[19] Morphological properties were examined by using a light microscope (Olympus BX43, Tokyo, Japan) and scanning electron microscope (VEGA IITESCNA, Brno, Czechia) after incubation on YIM 38# medium at 28 °C for 10 days. Carbon-source utilization was performed according to the methods of Shirling and Gottlieb[17] and Locci.[20] Growth at various pH, temperatures and NaCl contents were examined according to Xu *et al.*[21] by using YIM 38# medium as the basal

[1]Key Laboratory of Microbial Diversity in Southwest China, Ministry of Education, Yunnan Institute of Microbiology, Yunnan University, Kunming, China; [2]College of Life Science, Jiujiang University, Jiujiang, China; [3]Hongyun Honghe Tobacco (Group) Co., Ltd, Kunming, China; [4]Microbial Resource Center, KRIBB, Daejeon, Republic of Korea and [5]State Key Laboratory of Biocontrol and Guangdong Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-Sen University, Guangzhou, China
[6]These authors contributed equally to this work.
Correspondence: Professor W-J Li or Dr Y-Q Duan, Key Laboratory of Microbial Diversity in Southwest China, Ministry of Education, Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan 650091, China.
E-mail: wjli@ynu.edu.cn or dyanqing@aliyun.com
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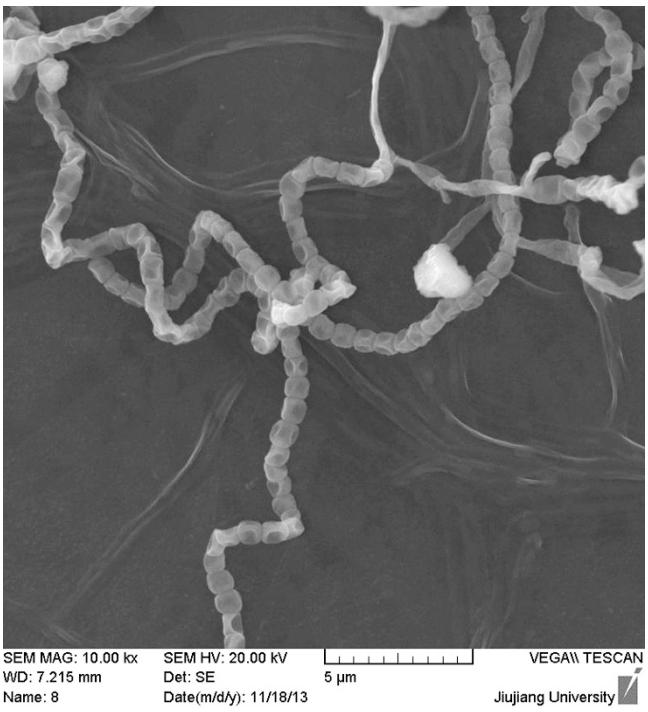

Figure 1 Scanning electron micrograph of spore chains of strain JXJ 0135^T after growth on YIM 38# medium at 28 °C for 7 days. Bar, 5 µm.

medium. Other phenotypic characteristics were determined by using standard procedures.[22,23]

Chemotaxonomy

The isomer of diaminopimelic acid and sugars of whole-cell hydrolysates were determined according to the procedures described by Hasegawa *et al.*[24] and Tang *et al.*[25] Phospholipids were extracted and analyzed according to published procedure.[26,27] Analysis of fatty acid was carried out according to the microbial identification system (Sherlock Version 6.1; MIDI database: TSSA6, MIDI, Inc., Newark, DE, USA). Menaquinones were extracted using the method of Collins *et al.*[28] and separated by HPLC.[29]

Molecular analysis

The sequence obtained was compared with available 16S rRNA gene sequences of cultured species from the EzTaxon-eserver (http://eztaxon-e.ezbiocloud.net/). Multiple alignments with sequences of the most closely related taxa by using CLUSTAL_X1.83.[30] Phylogenetic analyses were carried out by using neighbor-joining,[31] maximum-likelihood[32] and maximum-parsimony[33] methods. A phylogenetic tree was constructed by using the neighbor-joining tree-making algorithms[31] with MEGA version 5.0.[34] The topology of the phylogenetic tree was evaluated by using bootstrap analysis with 1000 replicates.[35] The G + C content of genomic DNA was determined using the HPLC method.[36] Levels of DNA–DNA hybridization was carried out according to Christensen *et al.*[37] and He *et al.*[38]

Anti-cyanobacterial activity

After being fermented in liquid medium (glucose 15 g, soybean powder 15 g, soluble starch 10 g, yeast extract 2 g, malt extract 2 g, peptone 2 g, NaCl 4 g, K_2HPO_4 0.4 g, $MgSO_4.7H_2O$ 0.5 g, $CaCO_3$ 2 g, H_2O 1000 ml, pH 7.8. $CaCO_3$ was added into the medium after adjustment of pH) for 10 days at 28 °C, 180 r.p.m., the fermentation broth of strain JXJ 0135^T was centrifuged and 2% (v:v) of the resultant supernatant was added into the cyanobacterial cultures, which were cultured in HGZ medium (Institute of Hydrobiology, Chinese Academy of Science, Wuhan, China) under an illumination of 30–50 µmol photon m^{-2} s^{-1} on a 12-h light–dark cycle at 25 °C. The anti-cyanobacterial activities of the fermentation supernatant were determined by the contents of chlorophyll *a* 3 days later, which were measured according to Chen *et al.*[39] The tested cyanobacteria included *Microcystis aeruginosa* FACHB-905, *M. wesenbergii* FACHB-1112, *M. viridis* FACHB-1284, *M. flos-aquae* FACHB-1285, *Oscillatoria planctonica* FACHB-708, *Aphanizomenon flos-aquae* FACHB-1171, *Anabaena flos-aquae* FACHB-1092, *Nostoc punctiforme* FACHB-252 and *O. tennuis* FACHB-247, which were all obtained from Institute of Hydrobiology, Chinese Academy of Sciences.

Table 1 Physiological characteristics of strains JXJ 0135^T and *S. scopuliridis* RB72^T

Characteristic	*1*	*2*
Spore chain	Coiling	None
Spore surface	Smooth	None
diffusible pigments	+	–
Temperatures (°C)	5–45	15–37
NaCl (w/v%)	0–4	4–7
pH	5–11	6–11
Catalase	–	ND
H_2S	–	ND
Milk coagulation	+	ND
Milk peptonization	+	ND
Nitrate reduction	+	+
Starch	+	+
Tyrosine	+	+
Urea	+	ND
Carbon utilization		
D-Fructose	+	–
D-Glucose	–	+
myo-Inositol	–	+
D-Mannose	–	+
D-Raffinose	–	+
Sodium malate	–	ND
L-Sorbose	+	–
Starch	+	+
Sucrose	+	–
Succinic acid	–	ND
D-Trehalose	–	ND
D-Xylose	–	+

Abbreviations: –, negative reaction; +, positive reaction; ND, no data.
Taxa: 1, strain JXJ 0135; 2, *S. scopuliridis* RB72^T.

RESULTS AND DISCUSSION

Strain JXJ 0135^T developed well-branched substrate and aerial mycelia on ISP2, ISP3, ISP5, Potato Dextrose Agar (PDA) and nutrient agar, with moderate growth on Czapek's agar and poor growth on ISP4. Soluble yellow pigments were produced on PDA, ISP2, ISP3 and ISP5. Spore chains were of spiral type, spores were elliptical and the spore surface was smooth (Figure 1). Detailed physiological characteristics are given in Table 1 and the species description.

LL-diaminopimelic acid was the diamino acid in the peptidoglycan of strain JXJ 0135^T. The whole-cell hydrolysates contained mannose, rhamnose, glucose and galactose. Phospholipids included diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol mannosides, phosphotidylinositol dimannoside, an unidentified aminophospholipid and an unknown phospholipid (Supplementary Figure S1). The menaquinones were MK-9(H_6) (69.0%), MK-9(H_8) (29.1%), MK-10(H_2) (0.5%), MK-10(H_4) (0.6%), MK-9(H_{10}) (0.6%) and MK-10(H_6) (0.2%). The major components of the fatty acids were anteiso-$C_{15:0}$ (26.6%), iso-$C_{16:0}$ (19.4%), anteiso-$C_{17:0}$ (15.2%), iso-$C_{15:0}$ (12.8%), $C_{16:0}$ (7.5%), iso-$C_{17:0}$ (6.3%), iso-$C_{14:0}$ (2.1%),

anteiso-$C_{17:0}$ *w9c* (1.8%), $C_{17:0}$ (1.3%), cyclo-$C_{17:0}$ (1.0%), $C_{17:1}$ *w8c* (0.6%), iso-H $C_{16:1}$ (0.5%), anteiso-$C_{13:0}$ (0.4%) and $C_{14:0}$ (0.4%). The G + C content of the genomic DNA from strain JXJ 0135^T was 69.3 mol%.

Analysis on the almost-complete 16S rRNA gene sequence (1516 bp) indicated that strain JXJ 0135^T belongs to the genus *Streptomyces* with the highest similarity to *S. scopuliridis* RB72^T (98.60%) and had lower 98.29% similarities with all the other type strains of the genus *Streptomyces*. Strain JXJ 0135^T formed a distinct clade with *S. scopuliridis* RB72^T by using three treeing methods (Figure 2). Stackebrandt and Ebers[40] recommended an increase of about 2% (from 97% to 98.7–99%) in the threshold for 16S rRNA gene sequence similarity used to determine the uniqueness of a novel isolate, provided that this level of difference in the sequences was supported by clear phenotypic differences. In this study, therefore, DNA–DNA relatedness experiments were only carried out between strain JXJ 0135^T and its most closely related type strain of *S. scopuliridis* RB72^T (= DSM 41917^T). The DNA–DNA relatedness with *S. scopuliridis* RB72^T was 41.2 ± 1.4, which supported the hypothesis that strain JXJ 0135^T belongs to different genomic species of the genus *Streptomyces*.

Moreover, many other phenotypic characteristics also distinguished strain JXJ 0135^T from its closest relatives (Table 1). Thus, based on the data in this study, we propose that strain JXJ 0135^T represents a novel species of the genus *Streptomyces*, and the name *Streptomyces lushanensis* sp. nov. is proposed.

Description of *Streptomyces lushanensis* sp. nov.

Streptomyces lushanensis (lu.shan'en.sis. N.L. adj. *lushanensis*. pertaining to Lushan Mountain, south China, from whence the strain was isolated).

Aerobic, Gram-positive actinomycete that forms well-branched substrate and aerial mycelia; aerial mycelia differentiate into spiral spore chains. Spores are elliptical with smooth surface. Aerial mycelia

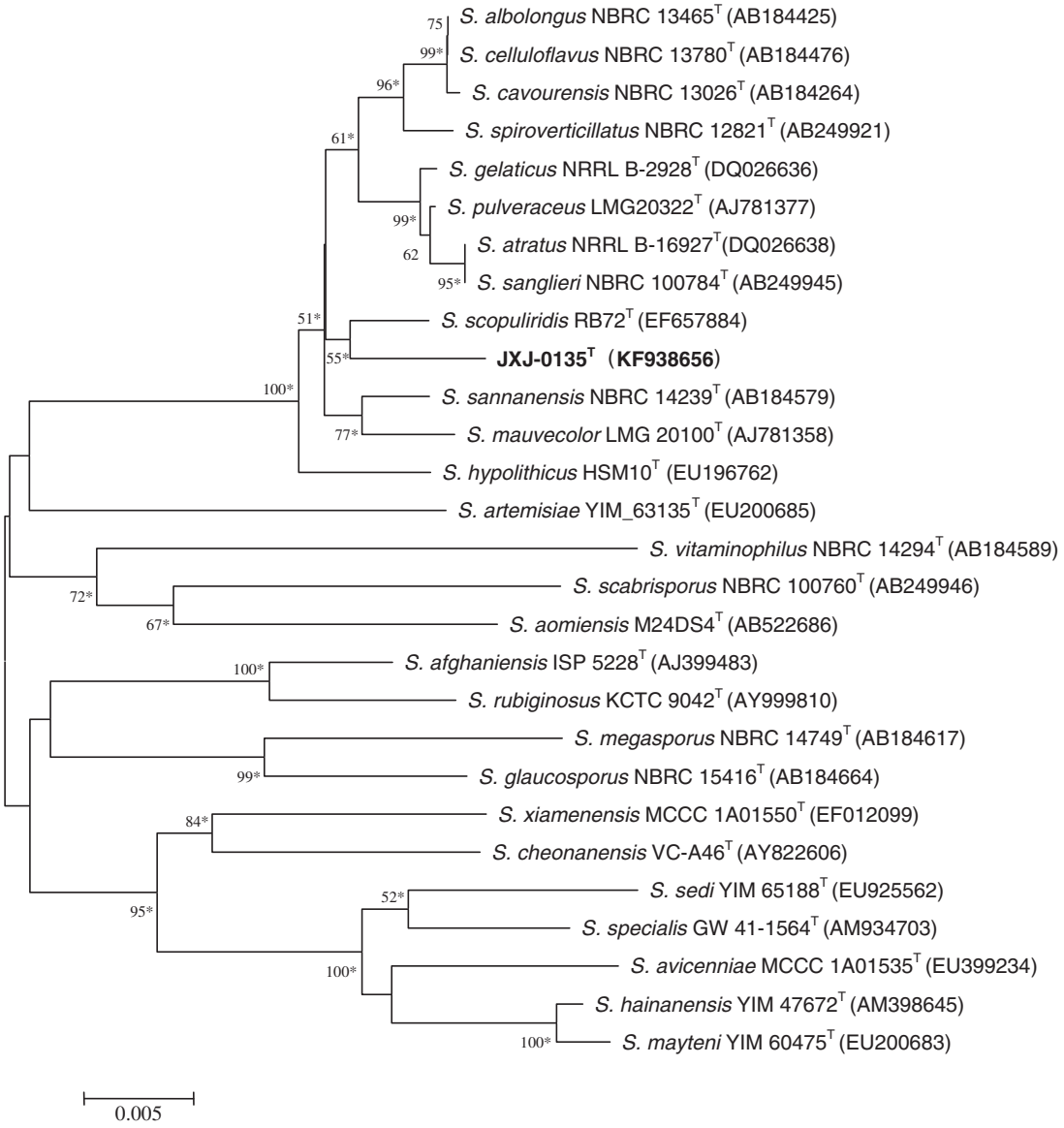

Figure 2 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of strain JXJ 0135^T and its closest relative species of the genus *Streptomyces*. Bootstrap values (expressed as percentages of 1000 replications) >50% are shown at the nodes. Asterisks indicate clades that were conserved when the maximum-likelihood method was used to construct the phylogenetic tree. Bar, 0.005 sequence divergence.

are white; vegetative mycelia are yellow-white. Yellow soluble pigments are produced. The pH, NaCl content and temperature range for growth are pH 5.0–11.0, 0–4% and 5–45 °C. Positive for hydrolysis of Tweens 40 and 80, nitrate reduction, gelatin, tyrosine, casein, milk coagulation, milk peptonization and hydrolysis of starch, but negative for urea, cellulose, catalase and H_2S. Utlilizes L-arabinose, D-galactose, D-fructose, lactose, maltose, L-sorbose and starch as sole carbon sources, but not D-glucose, myo-inositol, D-mannose, D-raffinose, sodium malate, sorbitol, sucrose, succinic acid, D-trehalose and D-xylose. L-Alanine, L-asparagine, L-histidine, hypoxanthine, L-glycine, L-methionine, L-phenylalanine, L-serine, L-tryptophan and L-tyrosine can be used as sole nitrogen sources, but not L-glutamine, L-lysine, L-arginine, L-threonine and L-isoleucine. The major fatty acids are iso and anteiso saturated fatty acids. The G + C content of the genomic DNA of the type strain is 69.3 mol%. It exhibits cyanolytic activities to many cyanobacteria such as *M. aeruginosa*, *M. wesenbergii*, *M. viridis*, *M. flos-aquae*, *O. planctonica*, *O. tennuis*, *A. flos-aquae*, *N. punctiforme* and *A. flos-aquae*.

The type strain, JXJ 0135^T (= DSM 42121^T = JCM 19628^T = KCTC 29261^T = KACC 17834^T = NRRL B-24994^T), was isolated from a soil sample collected in Lushan Mountain, south China.

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ACCESSION CODE

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JXJ 0135^T is KF938656.

1 Francis, G. Poisonous Australian lake. *Nature* **18,** 11–12 (1878).
2 Carmichael, W. in: *Advances in Experimental Medicine and Biology* (ed. Hudnell, H. K.) Ch. 4, 105–125 (2008).
3 Anderson, D. M. Turning back the harmful red tide. *Nature* **388,** 513–514 (1997).
4 Pan, G., Zou, H., Chen, H., Yuan, X. Z. & Zhang, M. M. Removal of harmful cyanobacterial blooms in Taihu lake using local soils. III. Factors affecting the removal efficiency and an in situ field experiment using Chitosan-modified local soils. *Environ. Pollut.* **141,** 206–212 (2006).
5 Wu, X. G., Joyce, E. M. & Mason, T. J. The effects of ultrasound on cyanobacteria. *Harmful Algae* **10,** 738–743 (2011).
6 Ozaki, K. *et al.* Lysis of cyanobacteria with volatile organic compounds. *Chemosphere* **71,** 1531–1538 (2008).
7 Choi, H. J., Kim, B. H., Kim, J. D. & Han, M. S. *Streptomyces neyagawaensis* as a control for the hazardous biomass of *Microcystis aeruginosa* (Cyanobacteria) in eutrophic freshwaters. *Biol. Control* **33,** 335–343 (2005).
8 Feng, Y. *et al.* Nanaomycin A methyl ester, an actinomycete metabolite: algicidal activity and the physiological response of *Microcystis aeruginosa*. *Ecol. Eng.* **53,** 306–312 (2013).
9 Safferman, R. S. & Morris, M. Evaluation of natural products for algicidal properties. *Appl. Microbiol.* **10,** 289–292 (1962).
10 Whyte, L. G., Maule, A. & Cullimore, D. Method for isolating cyanobacterial—lysing Streptomycetes from soil. *J. Appl. Bacteriol.* **58,** 195–197 (1985).
11 Yamamoto, Y., Kouchiwa, T. & Hodoki, Y. Distribution and identification of actinomycetes lysing cyanobacteria in a eutrotrophic lake. *J. Appl. Phycol.* **10,** 391–397 (1998).
12 Sigee, D. C. *et al.* Biological control of cyanobacteria: principles and possibilities. *Hydrobiologia* **395/396,** 161–172 (1999).
13 Yu, T. T., Zhang, B. H., Li, H. Q., Guo, Q. G. & Li, W. J. Alga-lysing activity of a strain of actinomycete to *Microcystis aeruginosa*. *J. Ecol. Rural Environ.* **27,** 58–63 (2011).
14 Bérdy, J. Bioactive microbial metabolites. *J. Antibiot.* **58,** 1–26 (2005).
15 Watve, M. G., Tickoo, R., Jog, M. M. & Bhole, B. D. How many antibiotics are produced by the genus *Streptomyces*? *Arch. Microbiol.* **176,** 386–390 (2001).
16 Jiang, Y. *et al. Streptomyces hainanensis* sp. nov., a novel member of the genus *Streptomyces*. *Int. J. Syst. Evol. Microbiol.* **57,** 2694–2698 (2007).
17 Shirling, E. B. & Gottlieb, D. Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.* **16,** 313–340 (1966).
18 Dong, X. Z. & Cai, M. Y. *Manual of Systematics and Identification of General Bacteria* (Science Press, Beijing, China, 2001).
19 Kelly, K. L. *Inter-Society Color Council–National Bureau of Standards Color-Name Charts Illustrated with Centroid Colors* (US Government Printing Office, Washington, DC, USA, 1964).
20 Locci, R. in *Bergey's Manual of Systematic Bacteriology* vol. 4 (eds Williams, S. T., Sharpe, M. E. & Holt, J. G.) 2451–2508 (Williams & Wilkins, Baltimore, MD, USA, 1989).
21 Xu, P. *et al. Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family Oxalobacteraceae isolated from China. *Int. J. Syst. Evol. Microbiol.* **55,** 1149–1153 (2005).
22 Goodfellow, M. Numerical taxonomy of some nocardioform bacteria. *J. Gen. Microbiol.* **69,** 33–80 (1971).
23 Williams, S. T. *et al.* Numerical classification of *Streptomyces* and related genera. *J. Gen. Microbiol.* **129,** 1743–1813 (1983).
24 Hasegawa, T., Takizawa, M. & Tanida, S. A rapid analysis for chemical grouping of aerobic actinomycetes. *J. Gen. Appl. Microbiol.* **29,** 319–322 (1983).
25 Tang, S. K. *et al. Zhihengliuella alba* sp. nov., and emended description of the genus *Zhihengliuella*. *Int. J. Syst. Evol. Microbiol.* **59,** 2025–2032 (2009).
26 Minnikin, D. E., Collins, M. D. & Goodfellow, M. Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. *J. Appl. Bacteriol.* **47,** 87–95 (1979).
27 Collins, M. D. & Jones, D. Lipids in the classification and identification of coryneform bacteria containing peptidoglycan based on 2,4-diaminobutyric acid. *Appl. Bacteriol.* **48,** 459–470 (1980).
28 Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. Distribution of menaquinones in actinomycetes and corynebacteria. *J. Gen. Microbiol.* **100,** 221–230 (1977).
29 Kroppenstedt, R. M. Separation of bacterial menaquinones by HPLC using reverse phase (RP18) and a silver loaded ion exchanger as stationary phases. *J. Liq. Chromatogr.* **5,** 2359–2387 (1982).
30 Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25,** 4876–4882 (1997).
31 Saitou, N. & Nei, M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4,** 406–425 (1987).
32 Felsenstein, J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* **17,** 368–376 (1981).
33 Fitch, W. M. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst. Zool.* **20,** 406–416 (1971).
34 Tamura, K. *et al.* MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28,** 2731–2739 (2011).
35 Felsenstein, J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39,** 783–791 (1985).
36 Mesbah, M., Premachandran, U. & Whitman, W. B. Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int. J. Syst. Bacteriol.* **39,** 159–167 (1989).
37 Christensen, H., Angen, Ø., Mutters, R., Olsen, J. E. & Bisgaard, M. DNA–DNA hybridization determined in micro-wells using covalent attachment of DNA. *Int. J. Syst. Evol. Microbiol.* **50,** 1095–1102 (2000).
38 He, L. *et al. Streptomyces jietaisiensis* sp. nov., isolated from soil in northern China. *Int. J. Syst. Evol. Microbiol.* **55,** 1939–1944 (2005).
39 Chen, Y. W., Chen, K. N. & Hu, Y. H. Discussion on possible error for phytoplankton chlorophyll-a concentration analysis using hot-ethanol extraction method. *J. Lake Sci.* **5,** 550–552 (2006).
40 Stackebrandt, E. & Ebers, J. Taxonomic parameters revisited: tarnished gold standards. *Microbiol. Today* **33,** 152–155 (2006).

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