INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Zhang *et al.*, *Int. J. Syst. Evol. Microbiol.* 2020;70:3139–3144

DOI 10.1099/ijsem.0.004145

MICROBIOLOGY SOCIETY

Serinicoccus hydrothermalis sp. nov., isolated from shallow-sea hydrothermal systems off Kueishantao Island

Qingfeng Zhang, Jianing Ye, Limei Yu, Dan Lin and Kai Tang*

Abstract

A Gram-stain-positive, non-flagellated, non-gliding, coccoid bacterial strain, designated JLT9^T, was isolated from the shallow-sea hydrothermal system off Kueishantao Island, Taiwan, ROC. Strain JLT9^T was aerobic, chemoheterotrophic and grew optimally at 35 °C, at pH 6.0 and in the presence of 2.5% (w/v) NaCl. Strain JLT9^T exhibited highest 16S rRNA gene sequence similarity to *Serinicoccus marinus* DSM 15273^T (98.83%). Phylogenetic trees based on 16S rRNA gene sequences revealed that strain JLT9^T belonged to the genus *Serinicoccus*, clustering with *Serinicoccus marinus* JC1078^T, *Serinicoccus profundi* MCCC 1A05965^T, *Serinicoccus sediminis* GP-T3-3^T and *Serinicoccus chungangensis* CAU9536^T. The digital DNA–DNA genome hybridization values between strain JLT9^T and the closest related strain *S. marinus* DSM 15273^T was 34.30%. The DNA G+C content was 72.43mol%. The dominant fatty acids were identified as iso-$C_{15:0}$ (41.4%) and iso-$C_{16:0}$ (24.7%). The polar lipids of strain JLT9^T comprised diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, three unidentified glycolipid and an unidentified phospholipid. The predominant isoprenoid quinone was MK-8 (H_4). The cell wall contained ornithine and serine, and no diaminopimelic acid. On the basis of phylogenetic data and several distinct phenotypic characteristics, strain JLT9^T represents a novel species of the genus *Serinicoccus*, for which the name *Serinicoccus hydrothermalis* sp. nov. is proposed. The type strain is JLT9^T (=CGMCC 1.15779^T=JCM 31502^T).

The genus *Serinicoccus*, a member of the family *Intrasporangiaceae*, was established by Yi *et al.* [1] with the description of *Serinicoccus marinus*. Members of the genus contain L-ornithine and L-serine as the diagnostic diamino acids in the cell-wall peptidoglycan [1]. Subsequently, three further species of the genus, *Serinicoccus profundi* isolated from deep-sea sediment, *Serinicoccus chungangensis* and *Serinicoccus sediminis* isolated from tidal flat sediment were described [2–4]. As an underexploited source of natural products, a new alkaloid along with five known compounds were isolated from *S. profundi* [5], while a potential antitumour agent was obtained from *Serinicoccus* sp. strain CNJ927 [6]. According to the results of analysis of the complete genome sequence of strain JLT9^T, a complete repertoire of genes for the oxidation of reduced sulfur compounds was found, which revealed the functional capability of sulfur metabolism [7]. In this study, we propose that strain JLT9^T represents a novel species of the genus *Serinicoccus*.

Seawater was collected on April 2014 from the shallow-sea hydrothermal system near Kueishantao Island (121° 57′ E, 24° 50′ N), offshore northeast of Taiwan, ROC, and served as a source for isolation of bacterial strains. Strain JLT9^T was isolated by the standard dilution plating technique on marine agar 2216 (MA; Difco) and was cultivated routinely at 30 °C. The isolate was preserved as glycerol suspensions (20% in sea water, w/v) at −80 °C. *Serinicoccus profundi* CGMCC 4.5582^T and *S. chungangensis* KCTC 19774^T were used as reference strains in the following study.

Cell morphology was observed using scanning electron microscope (JSM-6390LV, JEOL). Gliding motility was determined by the semi-solid agar puncture method [8]. The Gram reaction was investigated by using the Gram stain kit (HB8278, HopeBio) according to the manufacturer's instructions. The optimum temperature and pH for growth were examined in marine broth (MB; Difco) by monitoring the changes at OD_{600}. Growth at 0, 4, 13, 28, 30, 32, 35, 40 and

Author affiliations: [1]State Key Laboratory of Marine Environmental Science, Fujian Key Laboratory of Marine Carbon Sequestration, Xiamen University, Xiamen, PR China.
***Correspondence:** Kai Tang, tangkai@xmu.edu.cn
Keywords: *Serinicoccus*; shallow-sea hydrothermal system; polyphasic approach.
Abbreviations: ANI, average nucleotide identity; DDGH, DNA–DNA genome hybridization; MA, marine agar; MB, marine broth.
The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the complete genome sequence of strain JLT9^T are KU170959 and CP014989 respectively.
One supplementary table and four supplementary figures are available with the online version of this article.

45 °C was measured to determine the optimal temperature and temperature range for growth. The pH range for growth was tested by adjusting the final pH from 3 to 10 (in increments of 1 pH unit) with appropriate buffers (Na_2HPO_4/NaH_2PO_4 for pH 3.0–7.0 and $Na_2CO_3/NaHCO_3$ for pH 8.0–10.0). The optimum NaCl concentration for growth was tested at 0, 0.25, 0.50, 1.00, 1.50, 2.00, 2.50% and 3.00–15.00% (w/v) NaCl (intervals of 1%) in rich organic (RO) medium [9].

Aerobic/anaerobic culture tests were carried out by the semi-solid puncture method on anaerobic media (20 g hydrolysed casein, 5 g NaCl, 2 g sodium thioglycolate, 1 g hydroxymethanesulfinic acid sodium salt dihydrate, 15 g agar with 1 l H_2O) [8]. Catalase and oxidase activities were determined as described by Smibert and Krieg [10]. The ability to grow with carbohydrates or amino acids as sole sources of carbon and energy was examined by using Biolog GEN III MicroPlates [11]. Other phenotypic and enzymic characterizations were conducted using API 20NE and API ZYM kits (bioMérieux) according to the manufacturers' instructions. Antimicrobial susceptibility was tested by using the disc-diffusion plate method [12]. Susceptibility was tested to the following 15 antimicrobial agents (amounts per disc): penicillin G (10 U), ampicillin (10 μg), rifampicin (5 μg), streptomycin (10 μg), kanamycin (30 μg), gentamycin (10±2.5 μg), novobiocin (30 μg), tetracycline (30 μg), chloramphenicol (30 μg), erythromycin (15 μg), polymyxin B (300 U), lincomycin (2 μg), cephalothin(30 μg), neomycin (30 μg) and vancomycin(30 μg).

The 16S rRNA gene was amplified using a PCR with two universal primers 27F and 1492R [13]. The sequence of the 16S rRNA gene was compared with closely related sequences of reference organisms by the EzBioCloud service in GenBank database (NCBI) [14]. Phylogenetic trees were reconstructed via the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms, using the software MEGA version 7, after sequence alignment using the CLUSTAL W algorithm embedded into BioEdit [15–19]. The tree topology was evaluated by bootstrap analysis with 1000 replications [20]. The complete 16S rRNA gene sequence extracted from the genome data was also used to reconstruct the phylogenetic tree to ensure accuracy. The average nucleotide identity (ANI) values between strain JLT9^T and the closely related species was calculated using the ANI calculator (www.ezbiocloud.net/tools/ani) [14]. The *in silico* DNA–DNA genome hybridization (DDGH) values was calculated by the online Genome-to-Genome Distance Calculator GGDC2.1 (http://ggdc.dsmz.de/ggdc.php), with the alignment method of BLAST+ and recommended formula 2 [21]. Genome sequences of *S. marinus* DSM 15273^T, *S. profundi* CGMCC 4.5582^T and *S. chungangensis* KCTC 19774^T were downloaded from GenBank (GenBank accession numbers CP043808, CP042862, CP040887, respectively).

Cellular fatty acids analyses of strain JLT9^T and its reference strains were performed on cultures grown on MA at 25 °C for 2 days, carried out with the method described by Komagata and Suzuki [22]. The extracts were identified by the Microbial Identification System (MIDI) Sherlock version 6.0 and the library was TSBA6 6.00. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v) from dried cells and separated using the TLC method on silica gel [23]. Analysis was performed by HPLC [24], and the molecular weight was measured by mass spectrometry to identify the isoprenoid quinone. Polar lipids were extracted by a chloroform/methanol system and analysed by two-dimensional TLC as described previously [25, 26]. Preparation of the cell wall was performed using ultrasonic treatment in ice water bath and centrifugation, and the existence of the ornithine and serine was tested by employing TLC as described by Xu *et al.* [27]. The solvent system was methanol–pyrimidine–4 mol l^{-1} HCl–water (80:10:4:26, by vol.). The presence and absence of diaminopimelic acid were tested as described by Hasegawa *et al.* [28], using TLC on cellulose sheets instead of paper chromatography.

Cells of strain JLT9^T were Gram-stain-positive, non-flagellated, non-motile cocci and 0.5–0.8 μm in diameter (Fig. S1, available in the online version of this article). Colonies were yellow, circular, convex, smooth and 1 mm in diameter after 2 days (on MA at 30 °C) and reached the maximum diameter of 2.5 mm after 4 days. Strain JLT9^T was strictly aerobic. Growth occurred over a pH range of pH 3.0–10.0 (optimum, pH 6.0), at 4–35 °C (optimum, 35 °C) and in the presence of 0–15% (w/v) NaCl (optimum 2.5%). The strain was positive for catalase but negative for oxidase. In assays with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, trypsin, chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase and β-glucosidase were positive, cystine arylaminase was weakly positive. With API 20NE, positive for hydrolysis (β-glucosidase), hydrolysis (protease), β-galactosidase, glucose and the utilization of mannitol, maltose, malate, phenylacetic acid. Tested by the Biolog GENIII system, Tween 40, acetoacetic acid and acetic acid were oxidized. According to the antimicrobial susceptibility test, strain JLT9^T was sensitive to penicillin G (10 U), ampicillin (10 μg), rifampicin (5 μg), streptomycin (10 μg), novobiocin (30 μg), chloramphenicol (30 μg), erythromycin (15 μg), lincomycin (2 μg), cephalothin(30 μg), neomycin (30 μg) and vancomycin(30 μg) and resistant to kanamycin (30 μg), gentamycin (10±2.5 μg), tetracycline (30 μg) and polymyxin B (300 U).

The almost full-length 16S rRNA gene sequence (1307 bp) of strain JLT9^T was determined. One complete 16S rRNA gene sequence (1542 bp) from the genome data of strain JLT9^T extracted using ContEst16S [29] was compared with the partial 16S rRNA gene sequence obtained by Sanger sequencing, and the two sequences were found to have 99.08% similarity. According to the EzBioCloud result, the 16S rRNA gene sequence comparison to representative bacteria with validly published names indicated that strain JLT9^T shared the highest 16S rRNA gene sequence similarity with *S. marinus* DSM 15273^T (98.83%), *S. chungangensis* CAU 9536^T (97.16%), *S. sediminis* GP-T3-3^T (97.09%) and *S. profundi* MCCC 1A05965^T (96.92%). The phylogenetic

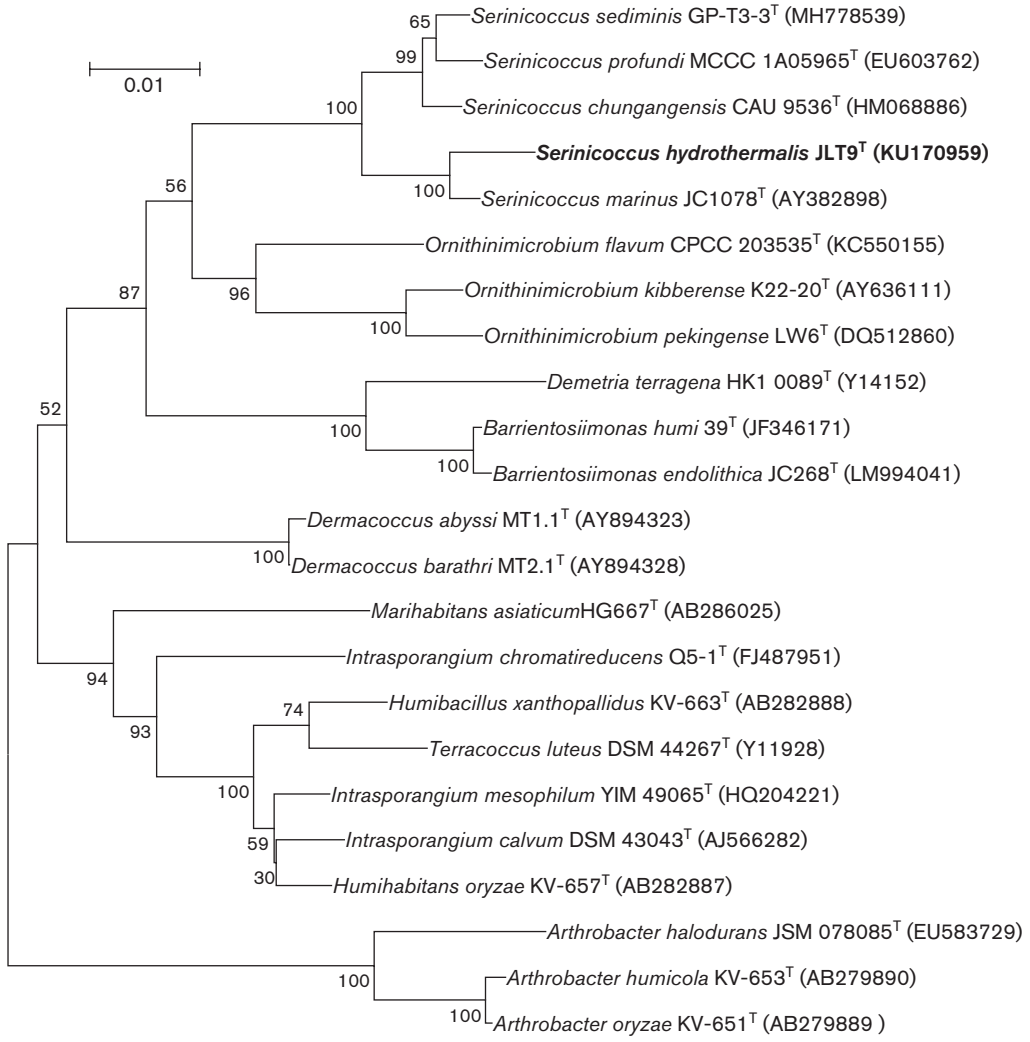

Fig. 1. A neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain JLT9^T and the type strains of the genus *Serinicoccus*. Bootstrap percentages were based on 1000 replications, the cluster of *Arthrobacter halodurans* JSM 078085^T, *Arthrobacter humicola* KV-653^T and *Arthrobacter oryzae* KV-651^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

trees indicated that strain JLT9^T formed a cluster with the genus *Serinicoccus* on the phylogenetic tree based on the neighbour-joining algorithm and showed that strain JLT9^T was closely related to *S. marinus* DSM 15273^T, *S. chungangensis* CAU 9536^T, *S. sediminis* GP-T3-3^T and *S. profundi* MCCC 1A05965^T as well (Fig. 1), which was also supported by phylogenetic analyses using the maximum-likelihood (Fig. S2) and maximum-parsimony algorithms (Fig. S3).

Strain JLT9^T has a circular chromosome of 3610932 bp, containing 3386 protein-coding genes, 45 tRNA genes and six rRNA genes. The DNA G+C content from genomic sequence data was 72.43 mol%, a value in the range reported for *Serinicoccus* species (Table 1) [7]. The ANI values of strain JLT9^T and three closely related species (*S. marinus*, *S. profundi* and *S. chungangensis*) were 87.94, 82.10 and 82.79% (Table 2). The values were below 95%, which is generally accepted for species delineation [30]. The digital DNA–DNA genome hybridization values between strain JLT9^T and closely related species (*S. marinus*, *S. profundi* and *S. chungangensis*) were 34.30, 24.70 and 25.10% (Table 2), which were below the standard cut-off value (70%) [31]. The results confirmed that strain JLT9^T represents a novel species of the genus *Serinicoccus*.

The chemotaxonomic data supported the result of the phylogenetic analysis. The dominant fatty acids were identified iso-$C_{15:0}$ (41.4%), iso-$C_{16:0}$ (24.7%), anteiso-$C_{15:0}$ (7.5%), summed feature 9 (iso-$C_{17:1}$ ω9*c*; 7.7%) and iso-$C_{17:0}$ (5.5%), similar to the major compositions of other strains of the genus *Serinicoccus* (Table S1). Major polar lipids of strain JLT9^T were diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, three unidentified glycolipids and an unidentified phospholipid (Fig. S4). The isoprenoid quinone found in strain JLT9^T was MK-8 (H_4), in line with members

Table 1. Differential characteristics between strain JLT9^T and the type strains of its closest phylogenetic relatives

Strains: 1, JLT9^T; 2, *Serinicoccus profundi* CGMCC 4.5582^T; 3, *Serinicoccus chungangensis* KCTC 19774^T; 4, *Serinicoccus marinus* JC1078^T; 5, *Serinicoccus sediminis* GP-T3-3^T. Abbreviations: +, Positive; -, negative; w, weakly positive.

Characteristic	1	2	3	4	5
Growth temperature range (°C)	4–35	10–35*	20–40†	10–35‡	4–45§
Growth pH range	3.0–10.0	6.0–11.0*	5.0–11.0†	6.0–11.0‡	4.0–12.0§
Growth in 15% (w/v) NaCl	+	−*	+†	−‡	+§
Nitrate reduction	−	+	+	+‡	−§
API ZYM					
Alkaline phosphatase	+	+	+	−‡	+§
Lipase (C14)	+	+	w	−‡	−§
Valine arylamidase	+	+	w	+‡	−§
Cystine arylaminase	w	+	+	−‡	−§
α-Galactosidase	+	−	+	−‡	−§
β-Glucosidase	+	w	+	w‡	−§
API 20NE					
Indole production	−	−	−	−‡	+§
Hydrolysis (β-glucosidase)	+	+	+	+‡	+§
Hydrolysis (protease)	+	+	+	+‡	+§
Mannitol utilization	+	−	+	+‡	+§
Biolog GENIII					
Pectin	−	−	+	−§	+§
Acetoacetic acid	+	+	−	+§	−§
Cellobiose	−	−	−	+‡	+§
Gelatin	−	−	−	+‡	+§
16S rRNA gene similarity to strain JLT9^T (%)	−	97.16	96.12	98.83	97.09
DNA G+C content (mol%)	72.4	72.0*	73.5†	72.0‡	72.9§
Diamino acids in peptidoglycan	Serine and ornithine	Serine and ornithine*	meso-Diaminopimelic acid†	Serine and ornithine‡	meso-Diaminopimelic acid§

*Data taken from Xiao *et al.* [2].
†Data taken from Traiwan *et al.* [3].
‡Data taken from Yi *et al.* [1].
§Data taken from Lee *et al.* [4].

Table 2. Genomic relatedness values between strain JLT9^T and *Serinicoccus* species

Strain	ANI value to JLT9^T (%)	DDGH value to JLT9^T (%)
Serinicoccus marinus DSM 15273^T	87.94	34.30
Serinicoccus chungangensis KCTC 19774^T	82.79	25.10
Serinicoccus profundi CGMCC 4.5582^T	82.10	24.70

of the genus *Serinicoccus*. The cell wall contained ornithine and serine, and no diaminopimelic acid (DAP) was found. However, ornithine and serine are typical components of cell-wall peptidoglycan of the genus *Serinicoccus*, which are found in *S. marinus* DSM 15273^T and *S. profundi* MCCC 1A05965^T, while the major peptidoglycan of *S. chungangensis* CAU9536^T and *S. sediminis* GP-T3-3^T is *meso*-DAP [1–4]. The differences of physiological and chemotaxonomic characterization between JLT9^T and other related type strains are given in Table 1.

The results suggested that strain JLT9^T represents a novel species of the genus *Serinicoccus*.

DESCRIPTION OF *SERINICOCCUS HYDROTHERMALIS* SP. NOV.

Serinicoccus hydrothermalis (hy.dro.ther.ma'lis. N.L. masc. adj. *hydrothermalis* from a hydrothermal area).

Cells are Gram-stain-positive, non-flagellated, non-motile strictly aerobic cocci and 0.5–0.8 µm in diameter. Colonies are yellow, circular, convex and smooth. The strain is oxidase-negative and catalase-positive. Growth occurs at 4–35 °C (optimum, 35 °C), pH 3.0–10.0 (optimum, pH 6.0) and with 0–15% (w/v) of NaCl (optimum, 2.5%). In API ZYM tests, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, trypsin, chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase and β-glucosidase are positive; cystine arylaminase is weakly positive. In API 20NE assays, positive for hydrolysis (β-glucosidase), hydrolysis (protease), β-galactosidase, glucose and the utilization of mannitol, maltose, malate and phenylacetic acid. In Biolog GENIII tests, Tween 40, acetoacetic acid and acetic acid are oxidized. The dominant fatty acids are identified as iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$, summed feature 9 (iso-$C_{17:1}$ ω9*c*) and iso-$C_{17:0}$. The cell wall contains ornithine and serine. The polar lipids comprise diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, three unidentified glycolipids and an unidentified phospholipid. The isoprenoid quinone is MK-8 (H_4).

The type strain is JLT9^T (=CGMCC 1.15779^T=JCM 31502^T), isolated from seawater in shallow-sea hydrothermal systems off Kueishantao Island, Taiwan, ROC. The DNA G+C content of the type strain is 72.43 mol%. The GenBank accession numbers for the 16S rRNA gene sequence and the whole genome shotgun sequence of strain JLT9^T are KU170959 and CP014989 respectively.

Funding information
This study was supported by the National Key Research and Development Program of China (2016YFA0601100), the National Natural Science Foundation of China project (91751207, 41776167, U1805242, 41676070), the Fundamental Research Funds for the Central Universities (20720190148), and the China Ocean Mineral Resources R and D Association (DY135-E2-1-04).

Conflicts of interest
Authors declare that there are no conflicts of interest.

References

1. Yi H, Schumann P, Sohn K, Chun J. *Serinicoccus marinus* gen. nov., sp. nov., a novel actinomycete with L-ornithine and L-serine in the peptidoglycan. *Int J Syst Evol Microbiol* 2004;54:1585–1589.
2. Xiao J, Luo Y, Xie S, Xu J. *Serinicoccus profundi* sp. nov., an actinomycete isolated from deep-sea sediment, and emended description of the genus *Serinicoccus*. *Int J Syst Evol Microbiol* 2011;61:16–19.
3. Traiwan J, Park M-H, Kim W. *Serinicoccus chungangensis* sp. nov., isolated from tidal flat sediment, and emended description of the genus *Serinicoccus*. *Int J Syst Evol Microbiol* 2011;61:1299–1303.
4. Lee DW, Yoo Y, Lee H, Kwon B-O, Khim JS *et al*. *Serinicoccus sediminis* sp. nov., isolated from tidal flat sediment. *Int J Syst Evol Microbiol* 2019;69:1998–2003.
5. Yang X-W, Zhang G-Y, Ying J-X, Yang B, Zhou X-F *et al*. Isolation, characterization, and bioactivity evaluation of 3-((6-methylpyrazin-2-yl)methyl)-1H-indole, a New alkaloid from a deep-sea-derived actinomycete *Serinicoccus profundi* sp. nov. *Mar Drugs* 2013;11:33–39.
6. Trzoss L, Fukuda T, Costa-Lotufo LV, Jimenez P, La Clair JJ *et al*. Seriniquinone, a selective anticancer agent, induces cell death by autophagocytosis, targeting the cancer-protective protein Dermcidin. *Proc Natl Acad Sci USA* 2014;111:14687–14692.
7. Han Y, Lin D, Yu L, Chen X, Sun J *et al*. Complete genome sequence of *Serinicoccus* sp. JLT9, an actinomycete isolated from the shallow-sea hydrothermal system. *Mar Genomics* 2017;32:19–21.
8. Dong XZ, Cai MY. Determination of biochemical properties. *Manual for the Systematic Identification of General Bacteria*. Beijing: Scientific Press; 2001. pp. 353–412.
9. Yurkov VV, Krieger S, Stackebrandt E, Beatty JT. *Citromicrobium bathyomarinum*, a novel aerobic bacterium isolated from deep-sea hydrothermal vent plume waters that contains photosynthetic pigment-protein complexes. *J Bacteriol* 1999;181:4517–4525.
10. Smibert RM, Krieg NR. Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (editors). *Methods for General and Molecular Microbiology*. Washington, DC: American Society for Microbiology; 1994. pp. 611–654.
11. Rüger H-J, Krambeck H-J. Evaluation of the Biolog substrate metabolism system for classification of marine bacteria. *Syst Appl Microbiol* 1994;17:281–288.
12. Fraser SL, Jorgensen JH. Reappraisal of the antimicrobial susceptibilities of *Chryseobacterium* and *Flavobacterium* species and methods for reliable susceptibility testing. *Antimicrob Agents Chemother* 1997;41:2738–2741.
13. Embley TM. The linear PCR reaction: a simple and robust method for sequencing amplified rRNA genes. *Lett Appl Microbiol* 1991;13:171–174.
14. Yoon S-H, Ha S-M, 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.
15. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
16. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
17. Fitch WM, Margoliash E. Construction of phylogenetic trees. *Science* 1967;155:279–284.
18. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
19. 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.
20. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
21. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
22. Komagata K, Suzuki K. Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 1987;19:161–207.
23. Tindall BJ. Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 1990;66:199–202.
24. Kroppenstedt RM. Fatty acid and menaquinone analysis of actinomycetes and related organisms. In: Goodfellow M, Minnikin DE (editors). *Chemical Methods in Bacterial Systematics (Society for Applied Bacteriology Technical Series vol. 20)*. New York: Academic Press; 1985. pp. 173–199.

25. **Collins MD**, **Goodfellow M**, **Minnikin DE**. Fatty acid, isoprenoid quinone and polar lipid composition in the classification of *Curtobacterium* and related taxa. *J Gen Microbiol* 1980;118:29–37.
26. **Kates M**. *Techniques of Lipidology*, 2nd ed. Amsterdam: Elsevier; 1986. pp. 106–107.
27. **LH X**, **WJ L**, **Liu ZH**, **Jiang CL**. *Actinomycete Systematic: Principle, Methods and Practice*. Beijing: Scientific Press; 2007. pp. 54–66.
28. **Hasegawa T**, **Takizawa M**, **Tanida S**. A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* 1983;29:319–322.
29. **Lee I**, **Chalita M**, **Ha S-M**, **Na S-I**, **Yoon S-H** *et al*. ContEst16S: an algorithm that identifies contaminated prokaryotic genomes using 16S RNA gene sequences. *Int J Syst Evol Microbiol* 2017;67:2053–2057.
30. **Kim M**, **Oh H-S**, **Park S-C**, **Chun J**. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* 2014;64:346–351.
31. **Wayne LG**, **Moore WEC**, **Stackebrandt E**, **Kandler O**, **Colwell RR**, **Brenner DJ**, **Grimont PAD**, **Trüper HG** *et al*. Report of the ad hoc Committee on reconciliation of approaches to bacterial Systematics. *Int J Syst Evol Microbiol* 1987;37:463–464.