J. Microbiol. Biotechnol. (2019), 29(12), 1969–1974
https://doi.org/10.4014/jmb.1812.12004

jmb

Labrenzia callyspongiae sp. nov., Isolated from Marine Sponge *Callyspongia elegans* in Jeju Island

So Hyun Park[1], Ji Young, Kim[2*], and Moon Soo, Heo[1*]

[1]*Department of Aquatic Life Medicine, Jeju National University, Jeju 63243, Republic of Korea*
[2]*Research Institute for Basic Science, Jeju National University, Jeju 63243, Republic of Korea*

Received: December 5, 2018
Revised: October 23, 2019
Accepted: October 23, 2019

First published online:
November 1, 2019

**Corresponding authors*
M.S.H.
Phone: +82-64-754-3473
Fax: +82-64-756-3493
E-mail: msheo@jejunu.ac.kr
J.Y.K.
Phone: +82-10-7253-2563
Fax: +82-64-756-3493
E-mail: jym1006@jejunu.ac.kr

Supplementary data for this paper are available on-line only at http://jmb.or.kr.

pISSN 1017-7825, eISSN 1738-8872

A Gram-staining-negative, aerobic, light brown pigment bacterium, designated strain CE80^T was isolated from marine sponge *Callyspongia elegans* in Jeju Island, Republic of Korea. Strain CE80^T grew optimally at 25°C, in the range of pH 5.0-11.0 (optimum 7.0-8.0), and with 1.0-5.0% NaCl (optimum 1-3% (w/v)). Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain CE80^T belonged to the genus *Labrenzia* and was closely related to *L. suaedae* YC6927^T (98.3%), *L. alexandrii* DFL-11^T (96.6%), *L. aggregata* IAM 12614^T (96.6%) *L. marina* mano18^T (96.5%) and *L. alba* CECT 5094^T (96.2%). The major fatty acids of strain CE80^T were $C_{18:1}$ ω7*c*, and summed feature. The polar lipids were diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmono-methylethanolamin, one unidentified aminolipid, one phospholipid and four unidentified lipids. The DNA G+C content of strain CE80^T was 55.9 mol%. The major respiratory quinone was Q-10. DNA-DNA relatedness between strain CE80^T and *L. suaedae* YC6927^T was 56.1±2.8%. On the basis of physiological and biochemical characterization and phylogenetic and chemotaxonomic analysis, strain CE80^T represents a novel species of the *Labrenzia*, for which the name *Labrenzia callyspongiae* sp. nov., is proposed. The type strain is CE80^T (=KCTC 42849^T =JCM 31309^T).

Keywords: Labrenzia, marine sponge, novel species, 16S rRNA

Introduction

The genus *Labrenzia*, established by Biebl *et al*. [1], belongs to the class *Alphaproteobacteria* and the family *Rhodobacteraceae*. At the time of writing, six species of the genus *Labrenzia* including the recently described species *L. suaedae* [2] and *L. salina* [3] have been identified, which were isolated from marine habitats and tidal flats, as dinoflagellates and halophytes, respectively. The members of genus *Labrenzia* are gram-negative, aerobic, and most species show the presence of glycolipid sulphoquinovosuldiacylglyceride (SQDG). Some species also synthesize bacteriochlorophyll *a* (BChl *a*). Previously, the genus *Agrobacterium* was reported by Stapp & Knosel [4] and Ruger & Hofle [5]; *Agrobacterium aggregatum* was described by Ahrens [6], and later the taxonomic position of the marine subdivision *Agrobacterium* was reassessed resulting in two species of the genus *Agrobacterium* getting transferred to the new genus *Stappia* as *Stappia aggregata* and *Stappia stellulata* [7] and finally reclassified as *Labrenzia aggregata* by Biebl *et al*. [1]. *Stappia alba* described by Pujalte *et al*. [8] and *Stappia marina* described by Kim *et al*. [9] were reclassified as *Labrenzia alba* and *Labrenzia marina*, respectively [1]. In the present study we determined the exact taxonomic position of strain ce80T by using a polyphasic characterization that included the determination of phenotypic, physiological and 16S rRNA gene sequence analysis, chemotaxonomic properties, and DNA-DNA hybridization.

Materials and Methods

Isolation

Strain CE80^T was isolated from marine sponge *Callyspongia elegans* collected at Jeju Island. To isolate marine bacteria, the

sponge sample of homogenized core tissue was 10-fold diluted with sterilized 0.85% (w/v) NaCl solution. Each dilution was inoculated onto marine agar 2216 (MA; Difco, USA) and incubated at 25°C for 7 days under aerobic conditions. The isolate was purified by repeated subculture and stored at −80°C as a suspension marine broth (MB; Difco) containing 20% (v/v) glycerol. The type strains of the most closely related species, *L. suaedae* KACC 13772 and *L. aggregate* KACC 15203^T were obtained from the Korean Agricultural Culture Collection (KACC); L. marina KCTC 12288^T was obtained from the Korean Collection for Type Cultures (KCTC); *L. alba* DSM 18320^T and *L. alexandrii* DSM 17067^T were obtained from the Deutsche Sammlung von Mikroorganismenund Zellkulturen (DSM).

16S rRNA Gene Sequencing and Phylogenetic Analyses

For 16S rRNA gene [10] sequencing, genomic DNA [11] was extracted from strain CE80^T and amplified by PCR with two universal primers, 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1522R (5′-AAGGAGGTGATCCAGCCGCA-3′). The amplified PCR product was cloned using the TOPO cloning kit (Invitrogen) and sequence analysis was performed (Genotech, Korea). Full sequence of the 16S rRNA gene was assembled using SeqMan software (DNASTAR) and we obtained the sequences for related taxa from GenBank and the EzTaxon-e server (www.ezbiocloud.net) [12]. The 16S rRNA gene sequences of strain CE80^T and related type strains were aligned by using CLUSTAL_X [13], while the BioEdit program [14] was used to edit gaps. Phylogenetic analysis was performed using the MEGA 6.0 software package [15] and phylogenetic trees were reconstructed based on the maximum-likelihood [16], maximum-parsimony [17] and neighbor-joining [18] methods through bootstrap analysis based on 1000 replicates [19]. Evolutionary distances were calculated using Jukes-Cantor model. The whole genome sequence of CE80T was performed by Macrogen (Republic of Korea) sequencing service. The sequencing library was prepared using TruSeq DNA PCR Fee kit according to the manufacturer's instructions. The genome sequence data were produced using the Illumina Hiseq 4000 platform and assembled *de novo* with SPAdes version 3.13.0 [20]. Also, the average nucleotide identity (ANI) was calculated using an ANI calculator (www.ezbiocloud.net/tool/ani) [21].

Morphology and Physiological, and Biochemical Characterization

The cell morphology of cell was observed by light microscopy (Nikon, Japan), scanning electron microscopy and transmission electron microscopy (SUPRA66VP, ZEISS). Gliding motility was determined according to Schaal [22]. Gram staining was conducted using the Gram Stain Kit (BD Science, USA) according to the manufacturer's instructions. Growth of strain CE80^T was evaluated on MA at different temperatures (5, 10, 15, 20, 25, 30, 35, 40, 45, and 50°C) for 5 days. The pH range for growth was determined in marine broth adjusted to pH 4.0-11.0 (0.5 pH unit intervals) using citric acid/sodium citrate buffer, KH_2PO_4/K_2HPO_4 and $NaHCO_2/Na_2CO_2$ as buffering systems. Tolerance to NaCl was tested in synthetic marine ZoBell medium (5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate, 15 g agar in 1L distilled water) (ZoBell, 1941) supplemented with various NaCl concentrations (0-10%, w/v, intervals 1%). Anaerobic growth was determined on MA at 25°C for 2 weeks under anaerobic condition (in an anaerobic jar with the Anaerobic Pack (Oxid)). Catalase and oxidase activity were tested by bubble formation in 3% hydrogen peroxide (H_2O_2) solution and using 1% (w/v) tetramethyl-*p*-phenylenediamine. Hydrolysis tests were performed using MA containing starch, casein, Tween (20, 40, 60, and 80), cellulose and DNase, respectively [23]. The enzyme activities and biochemical characteristics were tested by using API ZYM and API 20 NE kits (bioMerieux) according to the manufacturer's instructions. These tests were performed at 25°C for 5 days.

Chemotaxonomy

For cellular fatty acid analysis, strain CE80^T and one reference strain were harvested from MA after cultivation at 25°C for 5 days. Saponification, methylation and extraction of cellular fatty acids were performed by gas chromatography according to the protocol for the Sherlock Microbial Identification System (MIDI; version 6.1) and the TSBA6 database [24].

For analysis of the polar lipids, cell mass was harvested after incubation at 25°C for 5 days in MA and freeze-dried. Polar lipid analysis of strain CE80^T was carried out using two-dimensional TLC as described by Minnikin *et al*. [25]. The polar lipids were extracted and separated using chloroform/methanol/water (65:25:4, by volume) for the first dimension and chloroform/methanol/acetic acid/water (80:12:15:4, by volume) for the second dimension [25], while molybdophosphoric acid (for total lipids), ninhydrin (for lipids containing free amino groups), molybdenum blue (for phosphorus-containing lipids) and α-naphthol (for glycolipids) were used for the detection reagents [26].

Analysis of the respiratory quinones was performed by reversed-phase high-performance liquid chromatography [25].

Genomic Analyses

DNA G+C content of strain CE80^T was detected by using HPLC analysis [27] at the Korean Culture Center of Microorganisms (KCCM). DNA–DNA hybridization of strain CE80^T and type strain *L. suaedae* was performed with photobiotin-labelled DNA probes and microplate hybridization method by Ezaki *et al.* [28]. The experiment was with done five replications and the mean values were calculated for DNA–DNA relatedness.

To detect the presence of a gene related to production of bacteriochlorophyll a (BChl *a*), the *pufLM* gene was amplified using specific primers as described by Kim *et al*. [9].

Results and Discussion

Cells of strain CE80^T were gram-negative, aerobic, bright brown rods (0.56-0.74 μm × 1.5-2.7 μm) and exhibited gliding

motility. The growth occurred at 10–37°C (optimum 25°C) and pH 5.0–11.0 (optimum 7.5). The range of NaCl for growth was 1–5% (w/v) (optimum 1–3%). The detailed results of the morphological, physiological and biochemical characteristics are described and compared with type strains *Labrenzia suaedae* as listed in Table 1.

Table 1. Comparisons of phenotype characteristics of strain CE80^T and closely related type strains of the genus *Labrenzia*.

Characteristic	1	2	3	4	5	6
Growth at/with						
0% NaCl	-	+	-	-	+	-
10% NaCl	-	-	-	-	+	-
Hydrolysis of :						
DNA	+	-	+	+	-	-
Aesculin	-	+	+	+	+	+
API ZYM						
Cystine arylamidase	+	+	+	-	-	+
α-chymotrypsin	-	+	-	-	-	+
β-glucuronidase	-	+	-	+	-	-
α-glucosidase	-	+	+	+	-	+
β-glucosidase	-	+	-	-	-	-
N-acetyl-β-glucosamidase	-	+	+	+	-	-
α-mannosidase	-	+	-	+	-	-
α-fucosidase	-	+	-	-	-	-
API 20NE						
D-Glucose	-	+	-	-	-	+
D-Arabinose	-	+	-	-	-	+
D-mannose	-	+	-	-	-	+
D-Mannitol	-	+	-	-	-	+
N-acetyl-D-glucosamine	-	+	-	-	-	+
Gluconate	-	+	-	-	-	+
Malate	-	+	-	-	-	+
Citrate	-	+	-	-	-	+
Presence of pufLM genes	-	+	+	ND	-	+
Presence of SQDG	-	-	+	+*	+*	+*
DNA G+C content	55.9	58.5*	60*	ND	59*	56*

Strains: 1, Strain CE80^T (this study); 2, *L. suaedae* KACC 13772^T; 3, *L. marina* KCTC12288^T; 4, *L. alba* DSM 18320^T; 5, *L. aggregata* KACC 15203^T; 6, *L. alexandrii* DSM 17067^T. All strains are positive for motility, oxidase, catalase, reduction of nitrates to nitrites, hydrolysis of gelatin, assimilation of β-galactosidase, activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-galactosidase. All strains are negative for: hydrolysis of starch, casein, Tween (20, and 60), cellulose, indole production, glucose fermentation; arginine dihydrolase, assimilation of D-maltose, caprate, adipate, phenylacetate; lipase (C14) and naphthol-AS-BI-β D-glucuronide activities. *Data taken from Bibi *et al*. (2014).

The almost full-length 16S rRNA gene sequence of strain CE80^T (1,384 bp) was obtained. Based on phylogenetic analysis, strain CE80^T was shown to be most closely related to members of genus *Labrenzia* exhibiting highest 16S rRNA gene sequence similarity to *L. suaedae* YC6927^T (98.3%), *L. alexandrii* DFL-11^T (96.6%), *L. aggregata* IAM 12614^T (96.6%) *L. marina* mano18^T (96.5%) and *L. alba* CECT 5094^T (96.2%). A phylogenetic tree based on the neighbor-joining algorithm showed that strain CE80^T formed a phylogenetic lineage distinct from but related closely to type strain *Labrenzia suaedae* YC6927^T with 81% bootstrap support (Fig. 1).

The draft assembled genome size of strain CE80^T was 4,737,804 bp, contained seven contigs and an N50 length of 2,393,935 bp. The ANI value between strain CE80^T and type strain *L. suaedae* YC6927^T was 83.7%, which was lower than the standard cut-off of 95–96% [29] for species identity.

The presence of the *pufLM* genes can show ability to synthesize the photosynthetic reaction center. To confirm the presence *pufLM* genes of strain CE80^T, we used a specific primer and the gene was not found. This result shows that the photosynthetic reaction center may not be functional in this strain, which is found in other reported members such as *L. alexandrii*, *L. marina*, *L. suaedae*, and *L. salina* [1, 2, 3, 9].

Table 2. Cellular fatty acid compositions (%) of strain CE80^T and closely related type strains of the genus *Labrenzia*.

Fatty acid	1	2	3	4	5	6
Saturated						
$C_{10:0}$	tr	2.1	tr	tr	tr	tr
$C_{16:0}$	tr	2.1	tr	tr	tr	tr
$C_{18:0}$	4.7	tr	6.2	6.4	3.5	3.2
Unsaturated						
$C_{18:1}$ ω7c	66.8	66.4	55.9	70.5	69.9	67.7
$C_{20:1}$ ω7c	2.6	-	4.7	7.5	7.4	14.3
11-methyl $C_{18:1}$ ω7c	4.5	3.2	9.9	6.8	8.0	5.7
Cyclo $C_{19:0}$ ω8c	-	-	6.2	-	-	-
Hydroxy						
$C_{18:0}$ 3-OH	2.3	6.7	2.0	tr	tr	tr
Summed features						
2	10.3	8.8	3.1	3.7	4.1	3.7
3	tr	tr	8.5	-	tr	tr

Strains: 1, strain CE80^T (this study); 2, *L.suaedae* KACC 13772^T; 3, *L. marina* KCTC12288^T; 4, *L. alba* DSM 18320^T; 5, *L. aggregata* KACC 15203^T; 6, *L. alexandrii* DSM 17067^T. All data were obtained from this study. Values are percentages of total fatty acids (less than 2% are not show); tr, Traces (<2.0%); -, not detected. *Summed features represent groups of two or three fatty acids that cannot be separated by gas chromatography with the MIDI system. Summed features 2 comprised $C_{14:0}$ 3-OH and/or iso- $C_{16:1}$ I; 3, $C_{16:1}$ ω7*c* and/or iso- $C_{15:0}$ 2-OH.

The cellular fatty acids (>2 % of the total fatty acids) of strain CE80^T were $C_{18:1}$ ω7*c* (66.7%), summed feature 2 ($C_{14:0}$ 3-OH/ iso- $C_{16:1}$ I) (10.2%), $C_{18:0}$ (4.7%) 11-methyl $C_{18:1}$ ω7*c* (4.4%), $C_{20:1}$ ω7*c* (2.6%), and $C_{18:0}$ 3-OH (2.2%) (Table 2). The predominant fatty acids of strain CE80^T and *L. suaedae* were $C_{18:1}$ ω7*c* and summed features 2. The fatty acids $C_{18:1}$ ω9*c* and $C_{20:1}$ ω7*c* were present in strain CE80^T, but absent in *L. suaedae*. The predominant respiratory quinone of strain CE80^T was ubiquinone 10 (Q-10), which was in line with all members of the genus *Labrenzia*.

The predominant polar lipids of the strain CE80^T were diphosphatidylglycerol (DPG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylmonomethylethanolamin (PMME), one unknown aminolipid, one unknown phospholipid and four unknown lipids. These polar lipids profiles were the most similar pattern observed in all members of the genus *Labrenzia.* The presence of sulphoquinovosyldiacylglyceride (SQDG) was known to be characteristic in species of *Labrenzia*, but *L. suaedae* lacked SQDG. Similarly, SQDG

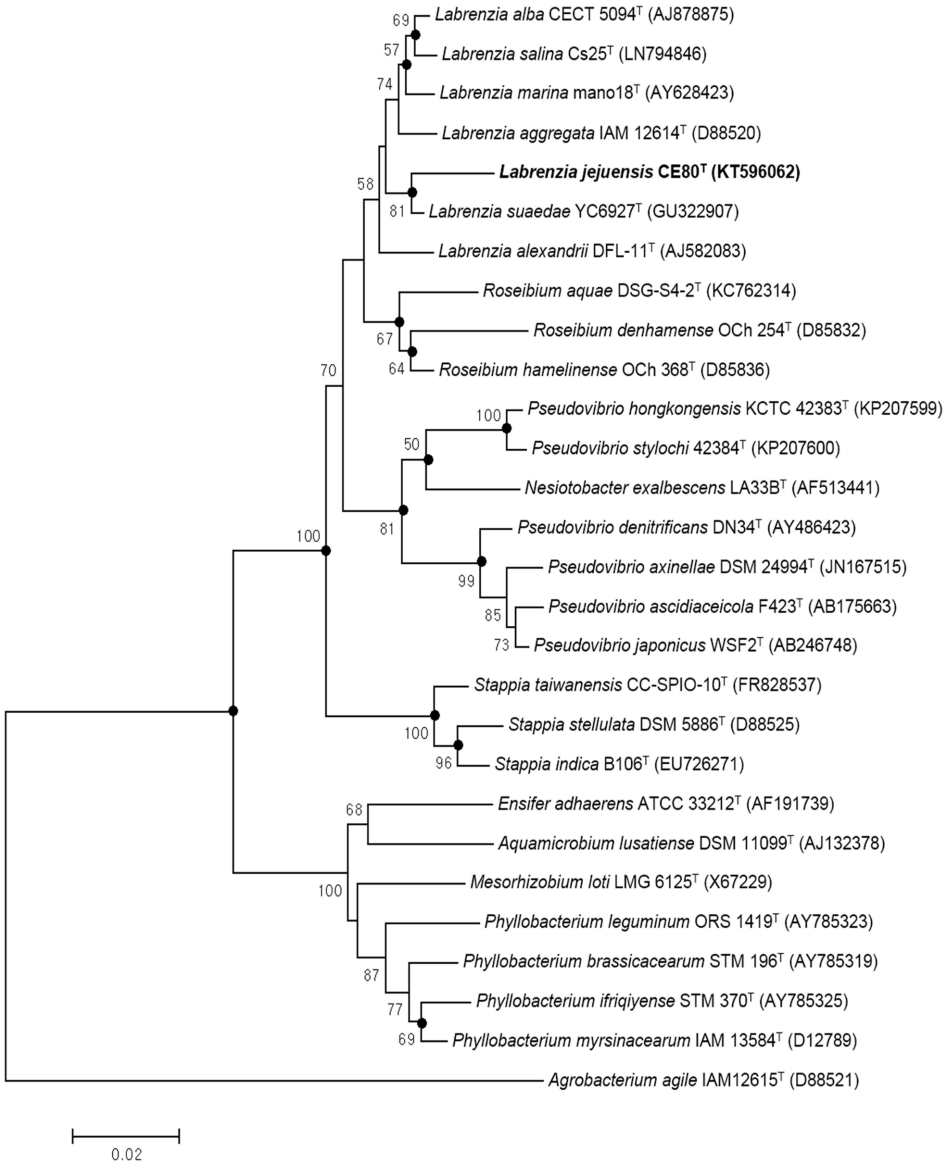

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, for strain CE80^T and genus *Labrenzia*.
Agrobacterium agile IAM 12615^T (D88521) was used as an outgroup. Numbers at branch nodes are bootstrap percentages based on 1000 resamplings; only values greater than 50% are shown. Filled circles indicate branches found in phylogenetic consensus trees generated with the Maximum-Likelihood and Maximum-Parsimony method. Bar, 0.02 substitutions per nucleotide position.

was detected in strain CE80^T in this study.

The DNA G+C content of strain CE80^T was calculated as 55.9 mol%, which is lower than the defined range values (58-63 mol%) reported for members of the genus *Labrenzia* [1, 2, 3, 8, 9]. The DNA-DNA hybridization value between strain CE80^T and type strain *L. suaedae* was 56.1 ± 2.8. This value was less than the 70% threshold value recommended for the delineation genomic species of novel strains by Wayne *et al.* [30]. Based on the results of phylogenetic, phenotypic, biochemical and chemotaxonomic analyses, strain CE80^T represents a novel species of the genus *Labrenzia*, for which the name *Labrenzia callyspongiae* sp. nov., is proposed.

Description of *Labrenzia callysponigae* sp. nov.

Labrenzia callyspongiae (cal.ly.spon'gi.ae. N.L. gen. n. *callyspongiae* of the sponge Callyspongia).

Strain CE80^T is gram-negative, aerobic, and exhibited gliding motility. The organisms were light-brown, rod-shaped, approximately 0.56-0.74 μm wide and 1.51-2.69 μm long and translucent after 3 days of incubation at 25°C on marine agar. Growth occurs at 10-37°C (the optimum temperature is 25°C), pH 5.0−11.0 (the optimum pH is 7.5) and in the 1-5% of NaCl (w/v) (optimum at 1−3%, w/v), respectively. The catalase and oxidase tests were positive. Nitrate reduced to nitrite. The strains also tested positive for hydrolysis of gelatin, Tween 80, urea and DNA but starch, casein, cellulose, aesculin, Tween 20, 40 and 60 tested negative. In the 20 NE tests, a positive result showed for assimilation of β-galactosidase, but indole production, glucose fermentation, arginine dihydrolase, assimilated of D-glucose, D-arabinose, D-mannose, D-mannitol, N-acetyl-D-glucosamine, D-maltose, gluconate, caprate, adipate malate, citrate and phenylacetate tested negative. In the ZYM tests, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucinearylamidase, valinearylamidse, cysteine arylamidase, trypsin, acid phosphatase, naphtol-AS-BI-phosphohydrolase, α-galactosidase were present, but lipase (C14), α-chymotrypsin, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosamidase, α-mannosidase and α-fucosidase were absent. The main fatty acids of strain CE80^T are $C_{18:1}$ ω7*c*, and summed feature 2. The polar lipids profile showed presence of diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmono-methylethanolamine, one unidentified aminolipid, one phospholipid and four unidentified lipids. The major respiratory lipoquinone was ubiquinone 10 (Q-10) and the DNA G+C content was 55.9 mol %.

The type strain CE80^T (=KCTC 42849^T =JCM 31309^T) was isolated from marine sponge *Callyspongia elegans* in Jeju Island, Republic of Korea.

GenBank Accession Number

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and whole genome sequence of *Labrenzia callyspongiae* CE80^T are KT596062 and WAJT00000000.

Acknowledgements

This research was supported by the Leading Human Resource Training Program of Regional Neo Industry through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2016H1D5A1911152 & 2017R1A2B4005688).

Conflicts of Interest

The authors have no financial conflicts of interest to declare.

References

1. Biebl H, Pukall R, Lünsdorf H, Schulz S, Allgaier M, Tindall BJ, *et al*. 2007. Description of *Labrenzia alexandrii* gen. nov., sp. nov., a novel alphaproteobacterium containing bacteriochlorophyll *a*, and a proposal for reclassification of *Stappia aggregata* as *Labrenzia aggregata* comb. nov., of *Stappia marina* as *Labrenzia marina* comb. nov., and of *Stappia alba* as *Labrenzia alba* comb. nov., and emended descriptions of the genera *Pannonibacter*, *Stappia* and *Roseibium*, and of the species *Roseibium denhamense* and *Roseibium hamelinense*. *Int. J. Syst. Evol. Microbiol*. **57:** 1095-1107.
2. Bibi F, Jeong JH, Chung EJ, Jeon CO, Chung YR. 2014. *Labrenzia suaedae* sp. nov., a marine bacterium isolated from a halophyte, and emended description of the genus *Labrenzia*. *Int. J. Syst. Evol. Microbiol*. **64:** 1116-1122.
3. Camacho M, Redondo-Gómez S, Rodríguez-Llorente I, Rohde M, Spröer C, Schumann P, *et al*. 2016. *Labrenzia salina* sp. nov., isolated from the rhizosphere of the halophyte *Arthrocnemum macrostachyum*. *Int. J. Syst. Evol. Microbiol*. **66:** 5173-5180.
4. Stapp C, Knösel D. 1954. Zur Genetik sternbildender Bakterien. Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt 2. **108:** 243-259 (in German).
5. Rüger HJ, Höfle MG. 1992. Marine star-shaped-aggregate-forming bacteria: *Agrobacterium atlanticum* sp. nov.; *Agrobacterium meteori* sp. nov.; *Agrobacterium ferrugineum* sp. nov., nom. rev.; *Agrobacterium gelatinovorum* sp. nov., nom.

rev.; and *Agrobacterium stellulatum* sp. nov., nom. rev. *Int. J. Syst. Bacteriol*. **42:** 133-143.
6. Ahrens R. 1968. Taxonomische Untersuchungen a sternbildenden *Agrobacterium*-Arten aus der westlichen Ostsee. *Kiel Meeresforsch*. **24:** 147-173 (in German).
7. Uchino Y, Hirata A, Yokota A, Sugiyama J. 1998. Reclassification of marine *Agrobacterium* species: proposals of *Stappia stellulata* gen. nov., comb. nov., *Stappia aggregata* sp. nov., nom. rev., *Ruegeria atlantica* gen. nov., comb. nov., *Ruegeria gelatinovora* comb. nov., *Ruegeria algicola* comb. nov., and *Ahrensia kieliense* gen. nov., sp. nov., nom. rev. *J. Gen. Appl. Microbiol*. **44:** 201-210.
8. Pujalte MJ, Macián MC, Arahal DR, Garay E. 2005. *Stappia alba* sp. nov., isolated from Mediterranean oysters. *Syst. Appl. Microbiol*. **28:** 672-678.
9. Kim BC, Park JR, Bae JW, Rhee SK, Kim KH, Oh JW, *et al*. 2006. *Stappia marina* sp. nov., a marine bacterium isolated from the Yellow Sea. *Int. J. Syst. Evol. Microbiol*. **56:** 75-79.
10. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol*. **173:** 697-703.
11. Wilson K. 1987. Preparation of genomic DNA from bacteria. In current protocols in molecular biology.
12. Yoon SH, Ha SM, Kwon SJ, Lim J, Kim Y, Seo H, *et al.* 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome. *Int. J. Syst. Evol. Microbiol*. **67:** 1613-1617.
13. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res*. **25:** 4876-4882.
14. Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser*. **41:** 95-98.
15. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol*. **30:** 2725-2729.
16. Felsenstein J. 1981. Evolutionary trees from DNA sequences: a maximum-likelihood approach. *J. Mol. Evol*. **17:** 368-376.
17. Kluge AG, Farris FS. 1969. Quantitative phyletics and the evolution of anurans. *Syst. Zool*. **18:** 1-32.
18. Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*. **4:** 406-425.
19. Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39:** 783-791.
20. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, *et al.* 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol*. **19:** 455-477.
21. Yoon SH, Lim JM, Kwon SJ, Chun J. 2017. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* **110:** 1281-1286.
22. Schaal, KP. 1986. Genus Acinomyces Harz 1877, 133AL. In Bergey's Manual of Systematic Bacteriology, vol. 2, pp. 1383-1418. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore; Williams & Wilkins.
23. Cowan ST, Steel KJ. 1965. Manual for the Identification of Medical Bacteria. Cambridge University Press: London.
24. Sasser M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids, MIDI Technical Note **101:** MIDI Inc.
25. Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, *et al*. 1984. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J. Microbiol. Methods* **2:** 233-241.
26. Komagata K, Suzuki K. 1987. Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol*. **19:** 161-207.
27. Tamaoka J, Komagata K. 1984. Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol. Lett*. **25:** 125-128.
28. Ezaki T, Hashimoto Y, Yabuuchi E. 1989. Fluorometric deoxyribonucleic acid deoxyribonucleic acid hybridization in microdilution 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.
29. Richter M, Rosselló-Móra R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. *Proc. Natl. Acad. Sci. USA* **106:** 19126-19131.
30. Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Moore LH, *et al*. 1987. International committee on systematic bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol*. **37:** 463-464.