Microbispora clausenae sp. nov., an endophytic actinobacterium isolated from the surface-sterilized stem of a Thai medicinal plant, Clausena excavata Burm. f.

Onuma Kaewkla1,2, Wilaiwan Koomsiri3,4, Arinthip Thamchaipenet4,5 and Christopher Milton Mathew Franco2,*

Abstract
An endophytic actinobacterium, strain CLES2T, was discovered from the surface-sterilized stem of a Thai medicinal plant, Clausena excavata Burm. f., collected from the Phujong-Nayoa National Park, Ubon Ratchathani Province, Thailand. The results of a polyphasic taxonomic study identified this strain as a member of the genus Microbispora and a Gram-stain-positive, aerobic actinobacterium. It had well-developed substrate mycelia, which were non-motile and possessed paired spores. A phylogenetic evaluation based on 16S rRNA gene sequence analysis placed this strain in the family Streptosporangiaceae, being most closely related to Microbispora bryophytorum NEAU-TX2-2T (99.4%), Microbispora camponoti 2C-HV3T (99.2%), Microbispora catharanthi CR1-09T (99.2%) and Microbispora amethystogenes JCM 3021T and Microbispora fusca NEAU-HEGS1-5T (both at 99.1%). The major cellular fatty acid of this strain was iso-C16:0 and major menaquinone was MK-9(H4). The polar lipid profile of strain CLES2T contained diphosphatidylglycerol, phosphatidylmethyl ethanolamine, phosphatidylglycerol and phosphatidyl glycerol dimannosides. These chemotaxonomic data confirmed the affiliation of strain CLES2T to the genus Microbispora. The DNA G+C content of this strain was 70 mol%. Digital DNA–DNA hybridization and average nucleotide identity BLAST values between strain CLES2T and M. catharanthi CR1-09T were 62.4 and 94.0%, respectively. The results of the polyphasic study allowed the genotypic and phenotypic differentiation of strain CLES2T from its closest species with valid names. The name proposed for the new species is Microbispora clausenae sp. nov. The type strain is CLES2T (=DSM 101759T=NRRL B-65340T).

The genus Microbispora belongs to the family Streptosporangiaceae [1]. This genus contains meso-diaminopimelic acid in the cell-wall peptidoglycan. The phospholipid is type IV, which contains phosphatidylcholine and unknown glucosamine-containing compounds, but no phosphatidylglycerol. Predominant menaquinones have nine isoprene units: MK-9(H2), MK-9(H4), MK-9(H0) and small amount of MK-9(H6). The fatty acid profile is a complex mixture of saturated, unsaturated, iso-, anteiso- and branched-chain fatty acids [2]. The genus Microbispora contains 12 species, which were discovered from various habitats such as soil, plant tissues and insects. Six species were isolated from soil, namely Microbispora rosea subsp. rosea, Microbispora rosea subsp. aerata [3], Microbispora coralline [4], Microbispora siamensis [5], Microbispora hainanensis [6] and Microbispora soli isolated from hot spring soil [7]. Five species were reported as endophytes which were isolated from different types of plant tissues namely Microbispora bryophytorum from moss [8], Microbispora catharanthi from Catharanthus roseus [9] and Microbispora tritici-radicis, Microbispora tritici and Microbispora fusca from wheat [10–12]. One species, Microbispora camponoti, was associated with a Japanese carpenter ant (Camponotus japonicas) [13].

Author affiliations: 1Department of Biology, Faculty of Science, Mahasarakham University, Maha Sarakham 44150, Thailand; 2Department of Medical Biotechnology, College of Medicine and Public Health, Flinders University, Bedford Park, SA 5042, Australia; 3Department of Genetics, Kasetsart University, Chatuchak, Bangkok 10900, Thailand; 4Omics Center for Agriculture, Bioresources, Food and Health, Kasetsart University (OmiKU), Bangkok 10900, Thailand; 5Department of Genetics, Kasetsart University, Chatuchak, Bangkok 10900, Thailand.

*Correspondence: Christopher Milton Mathew Franco, chris.franco@flinders.edu.au

Keywords: endophyte; genome; Microbispora clausenae; polyphasic taxonomy.

Abbreviations: ANIlb, Average Nucleotide Identity-BlAST; ANIlm, Average Nucleotide Identity-MUMmer algorithm; DAP, Diaminopimelic acid; dDDH, Digital DNA-DNA Hybridisation; dDDH, Digital DNA-DNA Hybridisation; GGDC, Genome to Genome Distance Calculator; GGDC, Genome to Genome Distance Calculator; HPDA, Half-strength Potato Dextrose Agar; ISP, International Streptomyces Project; LC-MS, Liquid Chromatography - Mass Spectrometry; NA, Nutrient Agar; tetra, tetra nucleotide signature correlation index; TLC, Thin Layer Chromatography; TYGS, Type strain Genomic Server; TYGS, Type strain Genomic Server.

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain CLES2T is KX394342. The GenBank/EMBL/DDJB accession number for the draft genome of strain CLES2T is JACBW000000000.

Three supplementary figures and one supplementary table are available with the online version of this article.

084518 © 2020 The Authors

This is an open-access article distributed under the terms of the Creative Commons Attribution License. This article was made open access via a Publish and Read agreement between the Microbiology Society and the corresponding author’s institution.
Strain CLES2T was isolated from the stem sample of a Thai medicinal plant (Clausena excavala Burm. f.) collected from the Phujong-Nayoa National Park, Ubon Ratchathani Province, Thailand (14.438954° N 105.344589° E), and processed within 4 h of collection [14]. Surface-sterilized stem tissue was placed onto VL70 medium containing a defined amino acid mixture and solidified with 0.8 % gellan gum [14, 15]. Strain CLES2T emerged as a small colony from the stem tissue after incubation for 2 weeks at 27 °C. Polyphasic taxonomy showed that this strain represents a novel species of the genus Microbispora, for which the name Microbispora clausenae sp. nov. is proposed.

Genomic DNA of strain CLES2T was extracted and used for 16S rRNA gene amplification and sequencing as described previously [14]. The 16S rRNA gene sequence of CLES2T was analysed using the EzTaxon-e server (www.ezbiocloud.net) [16]. The 16S rRNA gene sequences of representatives of all valid strains of the genus Microbispora available from GenBank/EMBL were subsequently aligned with strain CLES2T using clustal_x [17] with Nonomuraea cavernae SYSU K10005T as the outgroup. The phylogenetic trees were reconstructed based on the maximum-likelihood and neighbour-joining algorithms using the software package MEGA version X [18]. The Tamura–Nei model [19] was applied to the maximum-likelihood analysis using the Subtree-Pruning-Regrafting-Extensive (SPR level 5) program. The phylogenetic trees were reconstructed based on the maximum-likelihood and neighbour-joining algorithms using the software package MEGA version X [18]. The Tamura–Nei model [19] was applied to the maximum-likelihood analysis using the Subtree-Pruning-Regrafting-Extensive (SPR level 5) program. The neighbour-joining algorithm [20] was used according to Kimura's two-parameter model [21]. The topology of the tree

Table 1. Average nucleotide identity, digital DNA–DNA hybridization and Tetra values between strain CLES2T and its related species

| Strain/Analysis | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|-----------------|---|---|---|---|---|---|---|
| ANIb (%)        | 92.0 | 94.0 | 86.0 | 90.9 | 91.7 | 86.0 |
| ANIm (%)        | 94.1 | 95.6 | 89.8 | 92.9 | 93.7 | 89.8 |
| dDDH (%)        | 54.1 (C.I. model 51.4–56.8) | 62.4 (C.I. model 59.5–65.2) | 36 (C.I. model 33.6–34.5) | 48 (C.I. model 45.4–50.6) | 51.6 (C.I. model 48.9–54.2) | 36 (C.I. model 33.5–38.5) |
| Tetra (Z score) | 0.9982 | 0.9980 | 0.9942 | 0.9964 | 0.998 | 0.994 |
was evaluated by performing a bootstrap analysis [22] based on 1000 replications.

The phylogenetic trees clearly revealed that strain CLES2T was a member of the genus Microbispora (Figs 1 and S1, available in the online version of this article).

The 16S rRNA gene similarities of strain CLES2T (1452 nt) to its related species were 99.4% to M. bryophytorum NEAU-TX2-2T, 99.2% to both M. camponoti 2C-HV3T and M. catharanthi CR1-09T and 99.1% to both M. amethystogenes JCM 3021T and M. fusca NEAU-HEGS1-5T.

The results showed that strain CLES2T formed a different cluster with these closest type strains. The closest neighbours on both phylogenetic trees were M. bryophytorum NEAU-TX2-2T and M. camponoti 2C-HV3T, which shared 16S rRNA gene similarity at 99.4 and 99.2%, respectively. Other closest neighbours were M. amethystogenes JCM 3021T and M. fusca NEAU-HEGS1-5T. The type strain, M. catharanthi CR1-09T, which had 99.2% 16S rRNA gene similarity to and had the highest values of ANIb, ANIm and dDDH with strain CLES2T was located at the farthest distance when compared with the other four related species (Figs 1 and S1). According to 16S rRNA gene similarity and position of strain CLES2T on the phylogenetic trees, the four type strains M. bryophytorum NEAU-TX2-2T, M. catharanthi CR1-09T, M. camponoti 2C-HV3T and M. amethystogenes JCM 3021T were selected for comparative physiological and biochemical studies.

Genomic DNA for whole genome sequencing of strain CLES2T was extracted using GenElute (Sigma) and a short insert size library was prepared. The genome was sequenced by the HiSeq X-ten platform (Illumina; 2×150 bp paired-end reads) at the Beijing Genome Institute (BGI; Hong Kong). De novo assembly of the reads was achieved by using Unicycler (version 0.4.8; without long reads) [23].

The draft assembly of the genome of strain CLES2T was submitted to GenBank with the accession number JACBWX000000000. The phylogenetic tree of the genomes of strain CLES2T and its related taxa was reconstructed using the Type (strain) Genome Server (TYGS) [24, 25]. The tree inferred with FastME version 2.1.6.1 [26] from genome blast distance phylogeny (GBDP) and distances were calculated from genome sequences. The branch lengths were scaled in terms of GBDP distance formula d4.

The average nucleotide identity (ANI) values between strain CLES2T and four related species were evaluated with pairwise genome alignment by using the ANI-BLAST (ANIb) and ANI-MUMmer (ANIm) algorithms [27]. Correlation indexes of tetra-nucleotide signature (Tetra) were applied within the JSpecies Web Server [27, 28]. Digital DNA–DNA hybridization (dDDH) values were calculated by applying the Genome-to-Genome Distance calculator (GGDC 2.1; BLAST+ method) in which formula 2 (identities/HSP length) was applied to the incomplete draft genome [24].

The draft genome sequence of strain CLES2T was 7.25 Mb with a DNA G+C content of 70 mol%. The genome analysis resulted in the following ANIb and ANIm values between the draft genome of strain CLES2T and its related species:
the differential characteristics between strain CLES2\textsuperscript{T} and this novel species having an ANI value higher than 96%. Therefore, by Kim et al. Strain: 1, CLES2\textsuperscript{T}; 2, Microbispora bryophytorum NEAU-TX2-2\textsuperscript{T}; 3, Microbispora campanoti 2C-HV3\textsuperscript{T}; 4, Microbispora amethytesenes JCM 3021\textsuperscript{T}. Only fatty acids detected at more than 0.5% of the total are presented. –, Not detected. All the data are from this study.

| Fatty acid               | 1   | 2   | 3   | 4   |
|-------------------------|-----|-----|-----|-----|
| iso-C\textsubscript{14:0}| 1.2 | 2.5 | 2.6 | 2.4 |
| C\textsubscript{16:0}   | 0.6 | 1.4 | –   | –   |
| iso-C\textsubscript{15:0} | 12.4| 15.6| 18.1| 7.9 |
| antiso-C\textsubscript{15:0} | –  | 2.0 | 5.9 | 1.3 |
| C\textsubscript{16:1}   | 5.4 | 4.6 | 3.8 | 9.5 |
| iso-H-C\textsubscript{16:1}| 0.7 | –   | –   | –   |
| iso-C\textsubscript{16:0} | 43.3| 32.9| 35.3| 39.1|
| C\textsubscript{16:1} cis\textsuperscript{9} | 0.9 | 1.8 | –   | 0.8 |
| C\textsubscript{18:0}   | 1.6 | 3.1 | 1.8 | 3.6 |
| C\textsubscript{16:1} 10-methyl | 3.3 | 6.2 | 2.2 | 2.3 |
| iso-C\textsubscript{17:0} | 2.4 | 3.8 | 5.0 | 2.9 |
| antiso-C\textsubscript{17:0} | 0.6 | 2.0 | 1.8 | 2.1 |
| C\textsubscript{17:1} cis\textsuperscript{9} | 0.8 | 1.3 | 0.8 | 2.3 |
| iso-C\textsubscript{16:2}OH | 2.7 | 0.6 | 0.6 | 0.3 |
| C\textsubscript{18:0} | 0.9 | 1.1 | 1.7 | 5.3 |
| C\textsubscript{17:1} 10-methyl | 18.0| 14.5| 14.1| 13.3|
| iso-C\textsubscript{18:0} | 0.7 | 0.6 | 1.3 | 1.3 |
| C\textsubscript{18:1} cis\textsuperscript{9} | –   | 0.6 | –   | –   |
| C\textsubscript{18:0} | 1.1 | 1.1 | 1.1 | 0.7 |
| iso-C\textsubscript{18:2}OH | –   | –   | –   | 0.7 |
| C\textsubscript{18:1} 10-methyl TBSA | –   | 1.4 | 0.7 | –   |
| iso-C\textsubscript{20:3}I | –   | 0.6 | –   | –   |

The differential characteristics between strain CLES2\textsuperscript{T} and those of three related species, M. bryophytorum CR1-09\textsuperscript{T}, M. bryophytorum NEAU-TX2-2\textsuperscript{T} and M. fusca NBRC 13915\textsuperscript{T}, were 62.4, 54.1 and 36%, respectively (Table 1). These values are lower than the threshold of 70% used to define species [25, 30].

The phylogenetic tree based on the TYGS revealed the relationship between strain CLES2\textsuperscript{T} and the related type strains (Fig. 2). The result clearly showed that strain CLES2\textsuperscript{T} was positioned in a different node with its closest strains, M. bryophytorum NEAU-TX2-2\textsuperscript{T} and M. catharanthi CR1-09\textsuperscript{T}. Also, the phylogenetic tree of the genome showed that strain CLES2\textsuperscript{T} was placed in a different species cluster from these two type strains (Fig. 2).

Whole-cell sugar was analysed by the TLC method of Hasegawa et al. [31] and diaminopimelic acid (DAP) was identified by TLC using the method of Bousfield et al. [32]. The meso-isomer of DAP was detected from strain CLES2\textsuperscript{T} and the whole-cell sugar contained galactose, glucose, mannose and madurose, while the whole-cell sugars of the closest type strains, M. bryophytorum NEAU-TX2-2\textsuperscript{T}, were glucose and madurose [8] and M. catharanthi CR1-09\textsuperscript{T} contained galactose, glucose, madurose and a small amount of xylose [9].

The phospholipid pattern was determined as described by Minnikin et al. [33] and Komagata and Suzuki [34] using 5% ethanolic molybdropophosphoric acid, nihinhydrin, molybdenum blue reagent, α-naphtol, periodate-Schiff spray and Dragendorff reagent. The major lipids were diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol, dimannosides and four unknown and unidentified lipids that tested positive with ninhydrin and molybdenum blue reagents (Fig S2), which corresponds to phospholipid type IV [35].

Isoprenoid quinones were extracted and purified using the method of Minnikin et al. [34] and analysed by reverse phase LC-MS employing UV detection and electrospray mass spectrometry (ESI) according to Kaewkla and Franco [36].

Strain CLES2\textsuperscript{T} contained MK-9(H\textsubscript{4}) (43.4%) as the predominant menaquinone and MK-9(H\textsubscript{5}) (33.8%) and MK-9(H\textsubscript{6}) (23.1%), while M. catharanthi CR1-09\textsuperscript{T} contained MK-9(H\textsubscript{4}) (50%), MK9(H\textsubscript{5}) (34%), MK-9(H\textsubscript{6}) (11%) and MK-9(H\textsubscript{7}) (5%) [9] – the latter menaquinone was not detected in strain CLES2\textsuperscript{T}.

For the analysis of whole-cell fatty acids, strain CLES2\textsuperscript{T} and its three closest type strains were grown for 7 days at 27°C in tryptic soya broth (Oxoid) in an Erlenmeyer flask at 150 r.p.m. and harvested by centrifugation. Washed cells (100 mg) were saponified, methylated and extracted, and then the fatty acid methyl esters (FAMEs) were determined by following the protocols described by Microbial Identification Inc. (midi) [37]. The SACTIN6 method and Sherlock version 6.3 were used for analysis.

The whole-cell fatty acid pattern of strain CLES2\textsuperscript{T} was of the iso-branched type (Table 2). The major cellular fatty acids of this strain were iso-C\textsubscript{14:0} (43.3%), C\textsubscript{17:0} 10-methyl (18%) and iso-C\textsubscript{15:0} (12.4%), which was the same pattern found in all related type strains including M. catharanthi CR1-09\textsuperscript{T} [9].

The results of our chemotaxonomic study showed that strain CLES2\textsuperscript{T} was clearly different from M. catharanthi CR1-09\textsuperscript{T}.
The morphological characteristics of strain CLES2\textsuperscript{T} and the three closest type strains were studied on eight different media: International Streptomyces Project (ISP) 2, ISP 3, ISP 4, ISP 5, ISP 7 [38, 39], Bennett’s agar, half-strength potato dextrose agar and nutrient agar [39]. Colour determination was based on the Methuen Handbook of Colour [40]. Strain CLES2\textsuperscript{T} showed morphology belonging to the genus Microbispora, with a substrate mycelium that was well developed and an aerial mycelium formed well in some media. Cultural characteristics on different media are demonstrated in Table S1. Electron microscopy revealed that it formed paired spores (approximately 1×0.8 microns) with smooth surfaces (Fig. S3).

The physiological and biochemical characteristics of strain CLES2\textsuperscript{T} and its four closest type strains were studied. Acid production from 23 carbohydrates and decomposition of l-tyrosine, urea and aesculin were evaluated according to the methods of Gordon et al. [41]. Hydrolysis of starch, catalase production, assimilation of seven organic acids and utilization of four phenolic compounds as sole carbon source were described by Kurup and Schmitt [42]. Growth at different temperatures (4, 15, 27, 37, 45 and 55 °C), NaCl concentrations (1, 3, 5, 10, 15 and 20 %, w/v) and pH between pH 4 and 10 (in 1 pH unit intervals) were evaluated after incubation at 37 °C for 7–14 days on ISP 2 medium [42].

The physiological properties of strain CLES2\textsuperscript{T} and its closest neighbours, \textit{M. bryophytorum} NEAU-TX2-2\textsuperscript{T} and \textit{M. catharanthi} CR1-09\textsuperscript{T}, were significantly different (Table 3).

Strain CLES2\textsuperscript{T} could produce acid from fucose, maltose, myo-inositol and methyl d-glucopyranoside, but the closest type strain, \textit{M. bryophytorum} NEAU-TX2-2\textsuperscript{T}, could not. In contrast, the closest type strain could produce acid from...
meso-erythritol but strain CLES2T could not. Also, strain CLES2T could decompose L-tyrosine, assimilate malate and propionate, grow at 45°C and use pyridine and toluene as sole carbon sources but the closest type strain could not. On the other hand, the closest type strain could decompose urea, grow at 5 and 10% NaCl (w/v) but strain CLES2T could not.

Based on the data of ANIb and ANIm including dDDH, strain CLES2T shared the highest values with *M. catharanthi* CR1-09T. The physiology and biochemical properties of these two strains were compared. The result showed that strain CLES2T differed significantly from this reference strain. Strain CLES2T could not produce soluble pigment, but the reference strain could. The spore colour of strain CLES2T was reddish white on ISP 2 and ISP 7, but that of the reference strain was pinkish white. In addition, strain CLES2T could hydrolyse starch and skimmed milk, while the reference strain could not. Also, strain CLES2T grew weakly at pH 5 and 15°C, but the reference strain could not. They differed in terms of acid production and organic assimilation. Strain CLES2T produced acid from raffinose, rhamnose and trehalose and assimilated propionate and malate, but the reference strain could not. Also, strain CLES2T produced acid from *myo*-inositol and maltose, but the reference strain could only do so weakly. Also, strain CLES2T could use pyridine and toluene as sole carbon sources, but the reference strain could not.

Based on the results of this polyphasic study, strain CLES2T is proposed to represent a novel species of the genus *Microbispora*, named *Microbispora clausenae* sp. nov.

**DESCRIPTION OF MICROBISPORA CLAUSENAE SP. NOV.**

*Microbispora clausenae* (clau'se.nae. N.L. gen. n. clausenae of *Clausena*, pertaining to the plant from which the type strain was isolated).

Aerobic and catalase-positive. Grows between 15 and 45°C, but best growth occurs between 27 and 45°C. Grows well between pH 6.0 and 10.0 and in the presence of 3% (w/v) NaCl. Colonies are wrinkled with a dry surface. Substrate mycelium develops well on most media and aerial mycelium forms well on some media. Diffusible pigments are observed on ISP 2. The mycelium is extensively branched and forms paired spores. Paired rod-shaped spores (0.8×1.0 μm) are observed. Produces acid from arabinose, cellobiose, fucose, fructose, galactose, glucose, mannose, mannotol, maltose, *myo*-inositol, methyl D-glucopyranoside, sucrose, trehalose, rhamnose, ribose, salicin, trehalose and xylose, but not from ducitol, *meso*-erythritol or sorbitol. Assimilates acetate, citrate, lactate, malate and propionate, but not tartrate. Decomposes L-tyrosine, starch and skimmed milk, but not urea. Uses pyridine and toluene, but not phenol and benzene as a sole carbon source.

It is characterized by *meso*-diaminopimelic acid in its peptidoglycan layer and galactose, glucose, mannose and madurose as whole-cell sugars. Phospholipids are diphaspatidylglycerol, phosphatidylmethyl ethanolamine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol dimannosides and four unknown glycolipids. Major cellular fatty acids are iso-C18:0, iso-C₁₀:0 10-methyl and iso-C₁₅:0 MK-9(H₄), MK-9(H₁) and MK-9(H₃) are predominant menaquinones. The DNA G+C content of the type strain is 70 mol%.

The type strain, CLES2T (=DSM 101759T=NRRL B-65340T), is an endophytic actinobacterium isolated from the stem of a Thai medicinal plant, *Clausena excava*al Burm. f., which grows in Phu-Jong-Nayoa National Park, Ubon Ratchathani Province, Thailand. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CR1-09T is KX394342. The Whole Genome Shotgun project of strain CLES2T has been deposited at DDBJ/ENA/GenBank under the accession JACBWX0000000000. The version described in this paper is version JACBWX0000000000.
10. Han C, Tian Y, Zhao J, Yu Z, Jiang S et al. Microbispora triticiradicis sp. nov., a novel actinomycete isolated from the root of wheat (Triticum aestivum L.). Int J Syst Evol Microbiol 2018;68:3600–3605.
11. Han C, Zhao J, Yu B, Shi H, Zhang C et al. Microbispora tritici sp. nov., a novel actinomycete isolated from a root of wheat (Triticum aestivum L.). Antonie van Leeuwenhoek 2019;112:1137–1145.
12. Zhao J, Yu B, Han C, Cao P, Yu Z et al. Microbispora fusca sp. nov., a novel actinomycete isolated from the ear of wheat (Triticum aestivum L.). Int J Syst Evol Microbiol 2020;70:139–145.
13. Han C, Liu C, Zhao J, Guo L, Lu C et al. Microbispora camponoti sp. nov., a novel actinomycete isolated from the cuticle of Campanotus japonicus Mayr. Antonie van Leeuwenhoek 2016;109:215–223.
14. Kaewkla O, Franco CMM. Rational approaches to improving the isolation of endophytic actinobacteria from Australian native trees. Microb Ecol 2013;65:384–393.
15. Schoenborn L, Yates PS, Grinton BE, Hugenholtz P, Janssen PH. Liquid serial dilution is inferior to solid media for isolation of cultures representative of the phylum-level diversity of soil bacteria. Appl Environ Microbiol 2004;70:4363–4366.
16. Yoon SH, Ha SM, 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.
17. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 1997;25:4876–4882.
18. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 2018;35:1547–1549.
19. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 1993;10:512–526.
20. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 1987;4:406–425.
21. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 1980;16:111–120.
22. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985;39:783–791.
23. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicyclic: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 2017;13:e1005595.
24. Meier-Kolthoff JP, Göker M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 2019;10:2182.
25. 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.

26. Lefort V, Desper R, Gascuel O. FastME 2.0: a comprehensive, accurate, and fast distance-based phylogeny inference program. Mol Biol Evol 2015;32:2798–2800.
27. Richter M, Rosselló-Móra R, Oliver Glöckner F, Peoples J. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics 2016;32:929–931.
28. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci U S A 2009;106:19126–19131.
29. Kim M, Oh HS, Park SC, Chun J, HS O, Jongsik C. 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.
30. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR et al. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 2018;68:461–466.
31. Hasegawa T, Takisawa M, Tanida S. A rapid analysis for chemical grouping of aerobic actinomycetes. J Gen Appl Microbiol 1983;29:319–322.
32. Bousfield U, Keddie RM, Shaw S. Simple rapid methods of cell wall analysis as an aid in the identification of aerobic coryneform bacteria. Chemical method in bacterial Systematics. Technical Series 1985;20:221–236.
33. Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M et al. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 1984;2:233–241.
34. Komagata K, Suzuki K. Lipid and cell wall analysis in bacterial systematics. Methods Microbiol 1987;19:161–207.
35. Lechevalier MP, De Bievre C, Lechevalier H. Chemotaxonomy of aerobic actinomycetes: phospholipid composition. Biochim Syst Ecol 1977;5:249–260.
36. Kaewkla O, Franco CMM. Actinomycetospora caliliridis sp. nov., an endophytic actinobacterium isolated from the surface-sterilised root of an Australian native pine tree. Antonie van Leeuwenhoek 2019;112:331–337.
37. Sasser M. Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids, Technical note# 101; 2001.
38. Shirling EB, Gottlieb D. Methods for characterization of Streptomyces species. Boca Raton: CRC Press; 1993.
39. Atlas RM. Handbook of Microbiological Media. In: Parks LC (editor). Boca Raton: CRC Press; 1993.
40. Kornerup J, Wanscher H. Methuen Handbook of Colour. In: Pavey DF (editor), 3rd ed. Norfolk, Cox & Wyman Ltd; 1978.
41. Gordon RE, Barnett DA, Henderhan JE, Pang CH, Nocardia coeliaca, Nocardia autotrophica, and the Nocardin strain. Int J Syst Bacteriol 1974;24:54–63.
42. Kurup KV, Schmitt JA. Numerical taxonomy of Nocardia. Can J Microbiol 1973;19:1035–1048.
43. Farris JS. Estimating phylogenetic trees from distance matrices. Am Nat 1972;106:645–668.

Five reasons to publish your next article with a Microbiology Society journal

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as ‘excellent’ or ‘very good’.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at microbiologyresearch.org.