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Micromonospora musae sp. nov., an endophytic actinomycete isolated from roots of *Musa* species☆,☆☆

Nattakorn Kuncharoen[a], Takuji Kudo[b], Masahiro Yuki[b], Moriya Okuma[b], Auttaporn Booncharoen[a,c], Somboon Tanasupawat[a,*]

[a] Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand
[b] Japan Collection of Microorganisms, RIKEN BioResource Center, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan
[c] National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, Pathumthani 12120, Thailand

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ABSTRACT

Two novel actinobacterial strains, MS1-9^T and NGC1-4, were isolated from roots of *Musa* (ABB) cv. 'Kluai Namwa', collected from Chachoengsao province, and *Musa* (ABB) cv. 'Kluai Chang', from Suphan Buri province, Thailand, respectively. Comparative analysis of 16S rRNA gene (98.0 to 98.9% similarity), gyrase subunit B (*gyrB*) gene and whole-genome sequences emphasised that the strains MS1-9^T and NGC1-4 showed closely related with *Micromonospora peucetia* DSM 43363^T, *M. krabiensis* JCM 12869^T and *M. avicenniae* DSM 45758^T, respectively. Strains MS1-9^T and NGC1-4 contained *meso*-diaminopimelic acid in cell-wall peptidoglycan. Whole-cell sugars were glucose, xylose, mannose, and ribose. The acyl type of peptidoglycan was glycolyl. MK-10(H_6), MK-9(H_6), and MK-10(H_8) were presented as the major menaquinones. Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol were detected as predominant phospholipid profiles. The major cellular fatty acids consisted of *iso*-$C_{15:0}$, *anteiso*-$C_{15:0}$, *anteiso*-$C_{17:0}$, *iso*-$C_{17:0}$ and $C_{17:0}$. The DNA G+C content of strains MS1-9^T and NGC1-4 were 72.2 and 72.3 mol%, respectively. Draft genome sequences indicated by ANI values and digital DNA-DNA hybridisation analysis asserted that the strains MS1-9^T and NGC1-4 should be represented as a novel species within the genus *Micromonospora* for which the name *Micromonospora musae* sp. nov. is proposed. The type strain is MS1-9^T (=JCM 32149^T = TISTR 2659^T).

The genus *Micromonospora* was firstly proposed in 1923 by Ørskov and classified as the type genus of the family *Micromonosporaceae* within the phylum *Actinobacteria* [43]. At the time of writing, the genus *Micromonospora* consisted of 84 species and 7 subspecies with validly published names [33]. Members of this genus are aerobic, Gram-positive, filamentous bacteria which have the unique morphological characteristics including non-motile single spores borne directly on the tips of non-fragmented substrate mycelia, and aerial hyphae were absence [32]. *Micromonospora* strains are distributed in a wide range of habitats, for example, soils [11], sea sand [47], near-shore sediment [34], root nodules [49], roots of rice [17], medicinal plant's roots [21].

Micromonospora strains were commonly identified based on the 16S rRNA gene-based phylogeny and the use of protein-encoding of the housekeeping gene *gyrB* [15]. Nevertheless, they are not sufficiently divergent in the genus *Micromonospora* to discriminate the different species. Recently, the analysis of whole-genome sequences has been used for species delineation and providing more trustworthy information to describe the new isolates of the genus *Micromonospora* [4]. In this study, we reported the isolation and taxonomic status of novel endophytic *Micromonospora* strains MS1-9^T and NGC1-4 isolated from surface-sterilised roots of *Musa* (ABB) cv. 'Kluai Namwa' and *Musa* (ABB) cv. 'Kluai Chang' using a polyphasic taxonomic approach containing phenotypic, chemotaxonomic, and genotypic characteristics.

Healthy roots of *Musa* (ABB) cv. 'Kluai Namwa' and *Musa* (ABB) cv. 'Kluai Chang', were collected in Chacho-

Abbreviations: ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridisation; GGDC, Genome-to-Genome Distance Calculator; NJ, neighbour-joining; ML, maximum-likelihood; MP, maximum-parsimony.

☆ **Category:** New Taxa (Actinobacteria).

☆☆ The DDBJ accession number for the 16S rRNA gene and gyrase subunit B gene sequences of strains MS1-9^T and NGC1-4 are LC177516 and LC411966, and LC411965 and LC411967, respectively. The GenBank/EMBL/DDBJ accession numbers of the draft genome of strains MS1-9^T and NGC1-4, *Micromonospora costi* CS1-12^T and *Micromonospora endolithica* JCM 12677^T are RAZT00000000, RAZS00000000, RBAN00000000 and RBAK00000000.

* Corresponding author.
E-mail address: Somboon.T@chula.ac.th (S. Tanasupawat).

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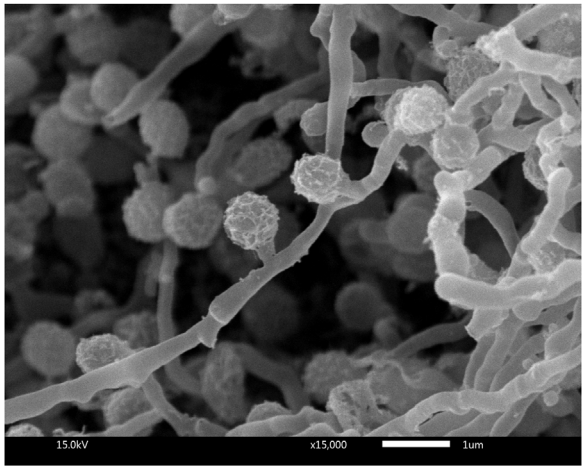

Fig. 1. Scanning electron micrograph showing strain MS1-9^T grown on yeast extract-malt extract agar medium at 30 °C for 21 days. Bars represent 1 μm.

engsao (13°39′34.6″N/101°04′33.4″E) and Suphan Buri (14°54′22.5″N/100°04′50″E) provinces, Thailand, respectively. Each sample was cross out, washed with running tap water and surface-sterilised following the modified method as described previously [21]. Each root was pestle in a sterile motar with 4% (w/v) sucrose solution. The homogenised samples were incubated at 60 °C for 10 min in a water bath then a 0.1 ml-portion of each resultant suspension was inoculated and spread on starch casein gellan gum (SCG) [22] supplemented with 25 μg nalidixic acid ml^{-1} and 50 μg cycloheximide ml^{-1}. After incubation at 30 °C for 21 days, colonies were transferred to purify on yeast extract-malt extract (ISP 2) agar medium [42]. The pure culture was maintained in 15% (v/v) glycerol solution at −80 °C and freeze-dried for long-term preservation.

Spore morphology was observed by scanning electron microscopy (JEOL, JSM-6610LV, Tokyo, Japan) after culturing on ISP 2 agar for 21 days at 30 °C. Cultural characteristics of strains MS1-9^T and NGC1-4 were determined after incubation at 30 °C for 2 weeks on ISP 2, ISP 3 (Difco), ISP 4 (Difco), ISP 5 (Nihon Pharmaceutical), nutrient agar (Difco) and Czapek's solution agar (Difco). The names and designations of the colonial colours and soluble pigments were assessed using the *Colour Harmony Manual* [14]. Growth at different of temperature (20–55 °C), pH (4–12) and NaCl (1–9%, w/v) concentration were evaluated using ISP 2 agar as the basal medium. The utilisation of carbohydrates as sole carbon sources was appraised using ISP 9 (Nihon Pharmaceutical) as the basal medium supplemented with a final concentration of 1% (w/v) of the carbon sources [42]. Starch hydrolysis, nitrate reduction, coagulation and peptonisation, gelatin liquefaction and H_2S production were tested on ISP 4 agar (Difco), ISP 8 broth (0.5% peptone, 0.3% beef extract, 0.1% KNO_3, pH 7.0), 10% skimmed milk (Difco), glucose-peptone-gelatine medium (2.0% glucose, 0.5% peptone, 20% gelatin pH 7.0) and ISP 6 agar (Nihon Pharmaceutical), respectively. Enzymatic activity was detected using the API ZYM (bioMérieux) kit.

Biomass for the chemotaxonomic and molecular systematic analyses was obtained from yeast-extract-dextrose broth (1% yeast extract, 1% glucose, pH 7.0) cultures grown for 7 days at 30 °C (200 rpm) and freeze dried. After extraction, as described by Staneck and Roberts [44], the isomers of cell-wall diaminopimelic acid (A_2pm) was determined by using thin-layer chromatography (TLC) and reducing sugars of whole-cell hydrolysates were analysed according to the HPLC procedure of Mikami and Ishida [29]. The *N*-acyl group of muramic acid in cell-wall peptidoglycan was detected following the method as described previously [50]. Cellular phospholipid profiles were extracted and identified using 2-dimensional TLC according to the method of Minnikin et al. [30]. Methyl esters of fatty acids were extracted by using the Sasser's protocol [40] and analysed by gas chromatography (MIDI, Sherlock Microbial Identification System, TSBA6 Sherlock Version 6.2B, USA). The presence of mycolic acid was prepared and monitored by the TLC method of Tomiyasu [48]. Isoprenoid quinones were extracted according to the method of Collins et al. [5], and were analysed by HPLC (Agilent 1100, Agilent, Santa Clara, CA, USA) and mass spectrometer (JEOL JMS-T, 100 L P) equipped with a Pegasil ODS column (Senshu, Tokyo, Japan), according to Tamaoka et al. [46].

Genomic DNA of strains MS1-9^T and NGC1-4 were prepared following the method as described by Kudo et al. [19] and purified using the AccuPrep Genomic DNA extraction kit (Bioneer Pacific, Australia) according to the manufacturer's instructions. The extracted chromosomal DNA has measured the purity and concentration by using Nanodrop ND-2000 UV–vis spectrophotometer. The 16S rRNA gene sequence was succeeded using the primers, 20 F (5′-GAGTTTGATCCTGGCTCAG-3′, positions 9–27) and 1530R (5′-GTTACCTTGTTACGACTT-3′, positions 1509-1492) as described by Suriyachadkun et al. [45]. The purified PCR products were sequenced on a DNA sequencer (Macrogen, Korea) using the universal primers as reported by Lane [23]. The extracted genomic DNA was also employed for DNA fingerprint based on BOX- and ERIC-PCR [51]. The 16S rRNA gene sequences were analysed using BioEdit software [13] and the sequence similarity values among the most phylogenetically related strains were done on EzBiocloud

Table 1
Differential phenotypic characteristics between strains MS1-9^T and NGC1-4 and their related type strains.

Characteristics	1	2	3	4	5	6	7	8
Starch hydrolysis	–	–	+	–	–	+	+	+
Peptonization	+	+	+	+	+	+	+	–
Coagulation	+	+	+	+	+	+	+	–
Gelatin liquefaction	–	–	–	–	–	–	–	+
Growth at 45 °C	–	–	–	–	–	–	+	+
Growth at pH 10.0	+	+	+	+	+	–	+	+
Utilisation of								
Adonitol	+	+	–	–	–	–	–	–
L-Arabinose	+	+	–	+	+	+	+	+
D-Cellobiose	+	+	+	+	+	w	+	–
α-Cyclodextrin	+	+	+	–	+	–	+	+
D-Fructose	+	+	w	w	+	+	w	w
D-Galactose	+	+	–	+	+	+	–	+
myo-Inositol	–	–	–	–	–	w	–	w
D-Mannose	+	+	+	+	+	+	–	+
D-Mannitol	w	w	–	+	–	w	–	–
D-Raffinose	–	–	–	+	+	–	–	+
D-Rhamnose	w	w	–	+	–	w	–	–
Salicin	+	+	–	–	–	–	–	w
Sucrose	+	+	+	+	+	+	–	+
API ZYM								
N-Acetyl-β-glucosaminidase	+	+	–	+	–	+	+	–
Acid phosphatase	+	+	+	+	–	+	+	+
Alkaline phosphatase	+	+	w	+	–	+	+	+
α-Chymotrypsin	+	+	+	+	–	+	+	+
Cystine arylamidase	w	w	–	–	–	–	–	w
Esterase (C 4)	+	+	+	w	+	+	w	w
Esterase Lipase (C 8)	w	w	+	w	+	w	w	w
α-Galactosidase	+	+	–	+	w	+	w	+
β-Galactosidase	+	+	–	+	+	+	+	+
β-Glucosidase	+	+	w	–	–	+	w	+
Lipase (C 14)	–	–	–	–	–	–	–	w
Naphthol-AS-BI-phosphohydrolase	+	+	+	+	w	–	w	w
Trypsin	+	+	+	+	w	+	+	+
Valine arylamidase	+	+	w	w	w	+	w	w

Strains: **1,** MS1-9^T; **2,** NGC1-4; **3,** *M. peucetia* JCM 12820^T; **4,** *M. avicenniae* JCM 31034^T; **5,** *M. endolithica* JCM 12677^T; **6,** *M. krabiensis* MA-2^T; **7**, *M. costi* CS1-12^T; **8**, *M. chersina* JCM 9459^T. All data were obtained from this study. +, Positive reaction; w, weak positive reaction; –, negative reaction.

Table 2
Description of *Micromonospora musae* sp. nov. according to Digital Protologue TA00874 assigned by the http://imedea.uib-csic.es/dprotologue/ website.

Taxonumber	TA00874
Species name	*Micromonospora musae*
Genus name	*Micromonospora*
Species epithet	*musae*
Species status	sp. nov.
Species etymology	mu'sae. N.L. gen. n. *musae*, of the botanical genus *Musa*
Author	Kuncharoen N, Kudo T, Masahiro Y, Ohkuma M, Booncharoen A, Tanasupawat S
Title	*Micromonospora musae* sp. nov., an endophytic actinomycete isolated from roots of *Musa* species.
Corresponding author	Somboon Tanasupawat
E-mail of the corresponding author	Somboon.T@chula.ac.th
Submitter	Nattakorn Kuncharoen
E-mail of submitter	n_kieng@hotmail.com
Designation of the type strain	MS1-9^T
Strain collection numbers	JCM 32149^T = TISTR 2659^T
16S rRNA gene accession number	LC177516
gyrB gene accession number	LC411965
Genome accession number [GenBank/RefSeq]	RAZT00000000
Genome status	Draft
Genome size (Mb)	7.06
GC mol%	72.2
Country of origin	Thailand
Region of origin	Chachoengsao province
Source of isolation	Roots of *Musa* (ABB) cv. 'Kluai Namwa'
Date of isolation	06 August 2015
Number of strains in this study	2
Source of isolation of non-type strains	Roots of *Musa* (ABB) cv. 'Kluai Chang', collected from Suphan Buri province
Growth medium, incubation conditions [Temperature, pH, and further information used for standard cultivation]	Yeast extract-Malt extract (ISP 2) agar, 30 °C, pH 7.0–8.0 without NaCl
Gram strain	POSITIVE
Cell shape	Filamentous
Motility	Nonmotile
Sporulation	Exospores
Mycelium	Substrate mycelia without aerial hyphae
Colony morphology	Substrate hyphae were brite-melon-yellow coloured on ISP 2 agar medium
Temperature range	28–30 °C
Temperature optimum	30 °C
Highest pH for growth	10.0
Lowest pH for growth	6.0
pH optimum	7.0–8.0
pH category	Neutrophile
Lowest NaCl concentration for growth	0 % (w/v)
Highest NaCl concentration for growth	4 % (w/v)
Salinity optimum	0–1 % (w/v)
Salinity category	Halotolerant
Relationship to O_2	Aerobe
Carbon sources used [specific compounds]	Adonitol, L-arabinose, D-cellobiose, α-cyclodextrin, D-fructose, D-galactose, D-glucose, D-mannose, D-mannitol, D-rhamnose, salicin, sucrose and D-xylose
Carbon sources not used [specific compounds]	Dextran, dulcitol, *myo*-inositol, D-raffinose
Positive test with API [APIZYM]	Acid phosphatase, Alkaline phosphatase, α-chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), α-galactosidase, β-galactosidase, β-glucosidase, α-glucosidase, *N*-acetyl-β-glucosamidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase
Negative test with API [APIZYM]	α-fucosidase, β-glucuronidase, lipase (C14) and α-mannosidase
Quinone type	MK-10(H_6), MK-9(H_6), MK-10(H_8), MK-9(H_8), MK-9(H_4) and MK-10(H_4)
Major fatty acids	*iso*-$C_{15:0}$, *anteiso*-$C_{15:0}$, *anteiso*-$C_{17:0}$, *iso*-$C_{17:0}$ and $C_{17:0}$
Peptidoglycan type	*meso*-diaminopimelic acid
Phospholipids pattern	Diphosphatidylglycerol, Phosphatidylethanolamine, Phosphatidylglycerol, Phosphatidylinositol

server (https://www.ezbiocloud.net/) [53]. The PCR amplification and sequencing of *gyrB* were carried out according to the method as described earlier [10]. Pairwise alignment similarities of the *gyrB* gene sequence of both strains and its phylogenetic relatives were calculated on GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A phylogenetic distance matrix was calculated with the Kimura-2-parameter model [16]. Similar tree topologies were monitored in the phylogeny constructed through the neighbour-joining [39], maximum-likelihood [7] and maximum-parsimony [9] algorithms using MEGA 7.0 [20]. The bootstrap values were calculated relied on 1000 replications [8]. Whole genome sequence analysis of strains MS1-9^T and NGC1-4 was implemented with an Illumina Miseq platform (Illumina, Inc., San Diego, US-CA) using 2 × 250 bp paired-end reads. A *de novo* assembling of the reads to contigs were accomplished by using SPAdes 3.12 [1]. The draft genomes of strains MS1-9^T and NGC1-4 are publicly available on the GenBank (accession numbers of RAZT00000000 and RAZS00000000, respectively). All genomes were annotated on Prokka software 1.12 [41] in line with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). A phylogenomic tree of strains MS1-9^T and NGC1-4 and their closest type strains was constructed by using TYGS web server (https://tygs.dsmz.de/) [27]. Average nucleotide identity (ANI) values of the strains MS1-9^T and NGC1-4 along with their closely related type strains were pairwise calculated using ANI-Blast (ANIb) and ANI-MUMmer (ANIm) algorithms [37] performed within the JSpeciesWS web service [38].

The Genome-to-Genome Distance Calculator (GGDC 2.1) with the BLAST + method [28] was used to evaluate the digital DNA-DNA hybridisation (dDDH). Results depended on recommended formula 2 (identities/HSP length), which is liberated of genome length and is thus prosperous against the use of incomplete whole genome sequences.

Based on the identical characteristics, it is visualised that strains MS1-9^T and NGC1-4 were classified into the genus *Micromonospora*. All strains produced extensively branched, non-fragmenting substrate mycelia, but no aerial hyphae was observed on all agar media. Single, non-motile, and spherical spores (0.8–0.9 μm in size) were borne on the substrate hyphae. The spore surface appeared rugose (Fig. 1). Colonies of strain MS1-9^T and NGC1-4 were brite–melon–yellow coloured on ISP 2 agar. Good growth was noticed on ISP 2 and ISP 4; moderate growth was monitored on ISP3 and ISP6, and poor growth was detected on ISP 5, ISP 7 and Czapek's solution agar media. Strains MS1-9^T and NGC1-4 produced clove–brown coloured diffusible pigment on ISP 7 agar. Cultural characteristics of strains MS1-9^T and NGC1-4 as well as the related type strains were given in Table S1. The growth was observed between pH 6.0 to 10.0 with an optimum at 8.0. Both strains grew well at 20–37 °C (optimally at 30 °C). Strains MS1-9^T and NGC1-4 grew well from 0 to 3% (w/v) NaCl, moderately on 4% (w/v), but failed to grow above 4% (w/v) NaCl. Other physiological and biochemical characteristics of strains MS1-9^T and NGC1-4 compared with the closest type strains were shown in Table 1 and the description of species (Table 2).

The *meso*-diaminopimelic acid was the diagnostic amino acid in cell-wall peptidoglycan of all strains. Glucose, xylose, mannose, and ribose was founded in the whole-cell hydrolysates. The acyl type of peptidoglycan was glycolyl. Mycolic acid methyl esters were absence. Diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), three unidentified phospholipids (PL1, PL2, PL3), two unidentified glycolipids (GL1, GL2), an aminophospholipid (APL) and three unidentified lipids (L1, L2, L3) were presented as the phospholipids profile (Fig. S1), corresponding to phospholipid type II of Lechevalier et al. [24]. Both strain MS1-9^T and NGC1-4 contained *iso*-$C_{15:0}$ (29.9, 31.2%), *anteiso*-$C_{15:0}$ (15.0, 14.9%), *anteiso*-$C_{17:0}$ (16.5, 12.2%), *iso*-$C_{17:0}$ (11.5, 9.0%), and $C_{17:0}$ (5.3, 10.7%) as predominant fatty acids according to fatty acid type 3b of Kroppenstedt [18]. Nevertheless, their quantities varied among the strains (Table 3). Strains MS1-9^T and NGC1-4 presented *iso*-$C_{10:0}$ (1.2, 2.6%) and *iso*-$C_{17:0}$ 3−OH (0.9, 1.2%) which are absent in all related type strains. The major menaquinones of strains MS1-9^T and NGC1-4 were MK-10(H_6) (31.5, 34.5%), MK-9(H_6) (22.9, 31.3%), MK-10(H_8) (19.1, 15.1%), and MK-9(H_8) (10.1, 9.3%) whereas MK-9(H_4) (9.0, 7.2%) and MK-10(H_4) (7.3, 2.7%) were minor components.

The analysis of 16S rRNA gene sequences indicated that the strains MS1-9^T (1400 nt) and NGC1-4 (1418 nt) are members of the genus *Micromonospora* of the family *Micromonosporaceae*. The 16S rRNA gene sequence pairwise analysis showed 100% similarity between the strain MS1-9^T and NGC1-4. Both exhibited 16S rRNA gene similarity value of 98.9% with *Micromonospora peucetia* DSM 43363^T and *M. costi* CS1-12^T subsequent by *M. chersina* DSM 44151^T (98.8%), *M. endolithica* DSM 44398^T (98.7%), *M. avicenniae* DSM 45758^T (98.4%) and *M. krabiensis* DSM 45344^T (=MA-2^T = JCM 12869^T) (98.0%). The results of the phylogenetic analysis using various tree-making methods were very similar (Fig. S2 and S3). The phylogenetic tree based on the neighbour-joining algorithm (Fig. 2) showed the position of strains MS1-9^T and NGC1-4 and the closest relatives. An extended NJ-tree including all *Micromonospora* species with validly published names is available as Supplementary Fig. S4. Although the two strains exhibited closely related with *M. peucetia* DSM 43363^T, an in-depth estimated at species level was performed

Table 3
Cellular fatty acids profiles (%) of strains MS1-9^T and NGC1-4 and their related type strains.

Fatty acid	1	2	3	4	5	6	7	8
Saturated fatty acids								
$C_{16:0}$	3.0	3.1	3.1	2.3	6.3	0.8	3.1	5.9
$C_{17:0}$	5.3	10.7	–	4.5	4.9	5.6	5.5	5.4
$C_{18:0}$	1.7	1.6	1.7	1.7	3.8	0.8	4.2	6.6
$C_{19:0}$	0.5	0.6	–	0.6	0.9	0.5	–	–
Unsaturated fatty acids								
$C_{17:1}\omega8c$	1.4	1.4	2.2	1.2	13.1	1.8	2.3	0.8
$C_{18:1}\omega9c$	0.7	0.5	5.5	–	7.6	–	1.7	0.9
Unsaturated branched fatty acids								
iso-$C_{15:1}$G	1.0	1.1	2.2	–	1.0	–	–	2.4
anteiso-$C_{17:1}\omega9c$	0.5	–	1.1	–	1.0	–	0.9	1.3
Branched fatty acids								
iso-$C_{10:0}$	1.2	2.6	–	2.5	–	–	–	–
iso-$C_{14:0}$	0.5	0.7	0.7	0.8	–	0.6	0.8	2.0
iso-$C_{15:0}$	29.9	31.2	20.1	25.6	13.8	20.3	15.0	17.0
anteiso-$C_{15:0}$	15.0	14.9	2.1	6.5	2.8	11.5	3.4	14.6
iso-$C_{16:0}$	6.5	4.1	26.8	19.3	7.4	15.9	27.1	18.4
iso-$C_{17:0}$	11.5	9.0	4.5	6.0	6.8	6.4	5.8	4.6
iso-$C_{17:0}$ 3-OH	0.9	1.2	–	–	–	–	–	–
anteiso-$C_{17:0}$	16.5	12.2	3.3	9.8	5.7	21.3	10.7	9.5
iso-$C_{18:0}$	–	–	–	–	–	0.6	1.1	0.7
iso-$C_{19:0}$	–	–	–	–	0.6	–	–	–
10-Methyl fatty acids								
10-Methyl $C_{17:0}$	0.8	0.9	4.6	9.3	7.8	8.6	6.7	0.7
10-Methyl $C_{18:0}$	–	–	6.7	0.9	4.2	1.0	1.1	–
Summed feature 3[a]	1.0	0.5	1.9	0.9	1.1	–	1.6	0.6
Summed feature 9[b]	1.8	1.5	7.3	3.2	8.1	1.9	2.7	0.9

The amount of fatty acid less than 0.5% in all strains was omitted.
Strains: **1,** MS1-9^T; **2,** NGC1-4; **3,** *M. peucetia* JCM 12820^T; **4,** *M. avicenniae* JCM 31034^T; **5,** *M. endolithica* JCM 12677^T; **6,** *M. krabiensis* MA-2^T; **7**, *M. costi* CS1-12^T; **8**, *M. chersina* JCM 9459^T. All data were obtained from this study.
[a] Summed feature 3 contained $C_{16:1}\omega6c$ or $C_{16:1}\omega7c$.
[b] Summed feature 9 comprised *iso*-$C_{17:1}\omega9c$.

with the phylogenetic analysis based on whole-genome (Fig. 3) and *gyrB* gene (Fig. S5) sequences indicated that the strains MS1-9^T and NGC1-4 showed phylogenetically closest with *M. avicenniae* DSM 45758^T and *M. krabiensis* DSM 45344^T, respectively. Therefore, based on the combination of the phylogenies relied on 16S rRNA gene, *gyrB* gene and whole-genome sequences, *M. avicenniae* DSM 45758^T (=JCM 31034^T), *M. costi* CS1-12^T, *M. krabiensis* DSM 45344^T (=MA-2^T = JCM 12869^T), *M. peucetia* DSM 43363^T (=JCM 12820^T), *M. chersina* DSM 44151^T (=JCM 9459^T) and *M. endolithica* DSM 44398^T (=JCM 12677^T) were considerably designed to the comparison of DNA-DNA homology.

Genetic profile of all strains based on BOX- and ERIC-PCR revealed that both strain MS1-9^T and NGC1-4 were not identical and could be clearly differentiated (Fig. S6). Draft genome sequences of strains MS1-9^T and NGC1-4, *M. endolithica* JCM 12677^T, and *M. costi* CS1-12^T, receiving from this study, were 7.06, 7.05, 6.97 and 7.18 Mb in size, respectively. The genome sequences of *M. peucetia* DSM 43363^T [4], *M. avicenniae* DSM 45758^T (FTNF00000000), *M. krabiensis* DSM 45344^T [4], and *M. chersina* DSM 44151^T (FMIB00000000) were obtained from the GenBank and were 7.36, 6.82, 7.07, and 6.68 Mb in size. *In silico* DNA G + C contents (mol%) of both new strains, *M. peucetia* DSM43363^T, *M. avicenniae* DSM 45758^T, *M. endolithica* JCM 12677^T, *M. krabiensis* DSM 45344^T, *M. costi* CS1-12^T and *M. chersina* DSM 44151^T were 72.2, 72.3, 72.5, 71.5, 72.8, 73.0 and 73.6 mol%, respectively. The genomic features of strains MS1-9^T and NGC1-4 and their type strains are given in Table 4. Average nucleotide identity values, ANIb and ANIm, of the genomes among both new isolates and all closest species were clearly lower than 95–96 % (Table 5) for the species delineation [37]. Strain MS1-9^T showed digital DNA-

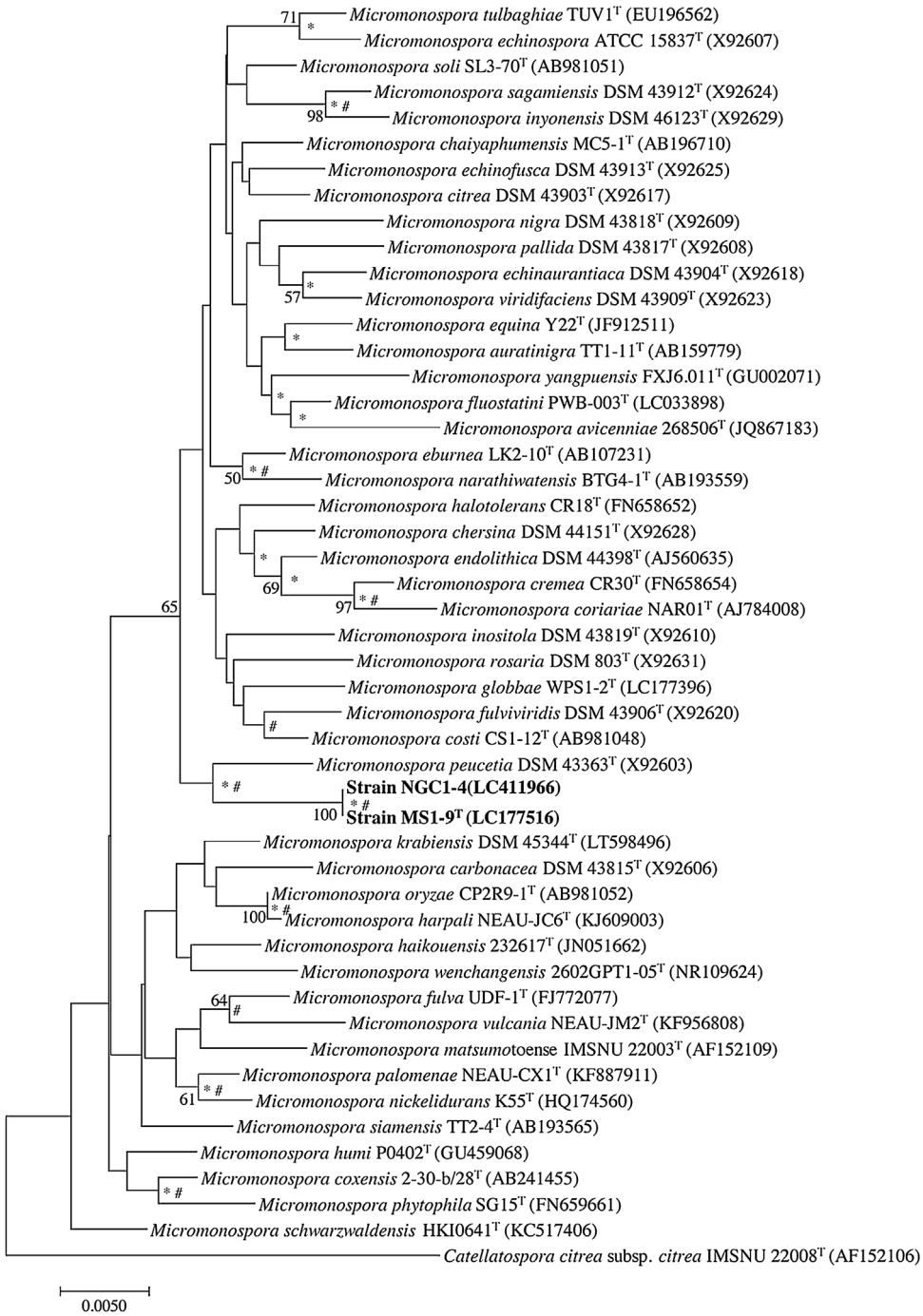

Fig. 2. Neighbour-joining phylogeny based on 16S rRNA gene sequences of strain MS1-9^T (1400 nt) and NGC1-4 (1418 nt) and the closest phylogenetically relatives. *Catellatospora citrea* subsp. *citrea* IMSNU 22008^T was used as an outgroup. Asterisk and sharp (*, #) indicate that the corresponding nodes were also recovered in the maximum-likelihood and maximum-parsimony trees, respectively. Numbers at branch nodes indicate levels of bootstrap support (%) derived from 1000 replications (only value over 50% are shown at). Bar, 0.005 substitutions per nucleotide position.

DNA hybridisation (dDDH) values of 38.7% (C. I. model 36.2–41.2%), 31.0% (C. I. model 28.6–35.5%), 28.9% (C. I. model 26.6–31.4%), 26.8% (C. I. model 24.4–29.3%), 25.9% (C. I. model 23.5–28.3%) and 25.7% (C. I. model 23.4–28.2%) with *M. avicenniae* DSM 45758^T, *M. costi* CS1-12^T, *M. krabiensis* DSM 45344^T, *M. peucetia* DSM 43363^T, *M. chersina* DSM 44151^T and *M. endolithica* JCM 12677^T, respectively (Table 5). The dDDH values were lower to the recommended threshold 70% for species delineation [12] supports the proposal of strains MS1-9^T and NGC1-4 representing a new species within the genus *Micromonospora*.

Draft genomes of strains MS1-9^T and NGC1-4, *M. avicenniae* DSM 45758^T, *M. costi* CS1-12^T, *M. krabiensis* DSM 45344^T, *M. peucetia* DSM 43363^T, *M. chersina* DSM 44151^T and *M. endolithica* JCM 12677^T were evaluated using the antiSMASH server [3] to detect putative biosynthetic gene clusters. The genomes of strains MS1-9^T and NGC1-4 were found to encode for terpene and corresponding residues which made up the catalytic triad found in RppA, a terpene comprised in the biosynthesis of sioxanthin as generally found in the genomes of *Salinispora tropica*, *S. arenicola*, *S. pacifica* [36] and all of *Micromonospora* strains [4]. The terpene of strains MS1-9^T and NGC1-4 showed 91, 82 and 80%

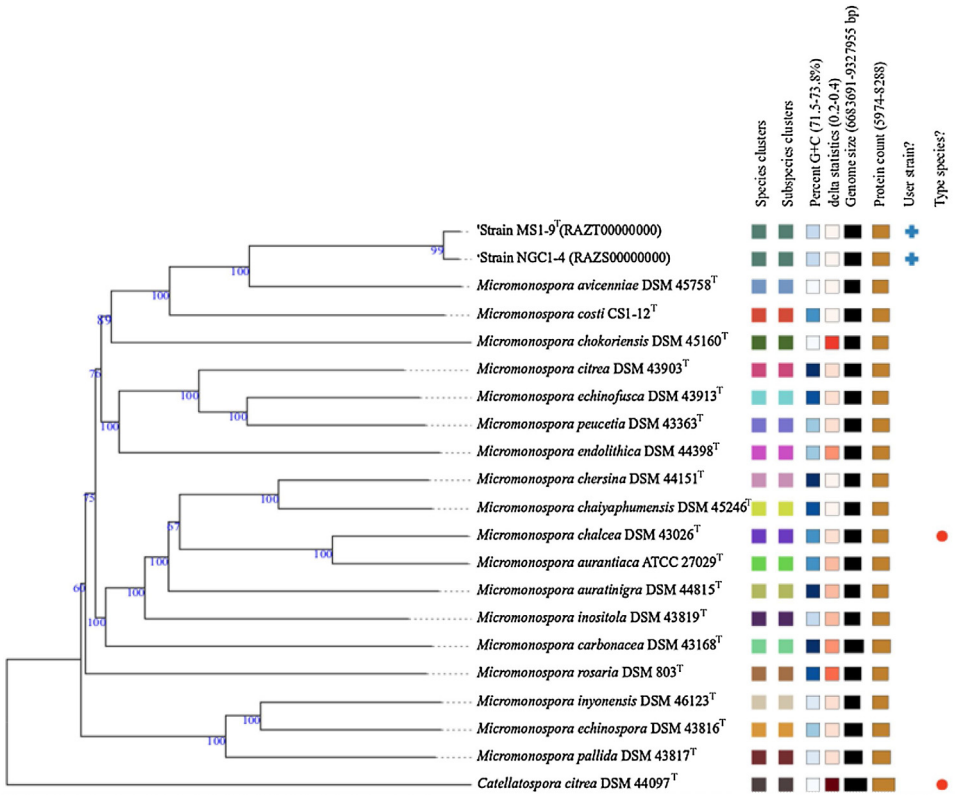

Fig. 3. The phylogenomic tree of strains MS1-9^T and NGC1-4 and their related type strains of the genus *Micromonospora* obtained from TYGS. Tree inferred with FastME 2.1.6.1 [26] from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d_5. The numbers above branches are GBDP pseudo-bootstrap support values from 100 replications, with an average branch support of 92.6%. The tree was rooted at the midpoint [6].

Table 4
Genome statistics of strains MS1-9^T and NGC1-4 and their related type strains.

Feature	1	2	3[b]	4[a]	5	6[b]	7	8[a]
Accession no.	RAZT00000000	RAZS00000000	FMIC00000000	FTNF00000000	RBAK00000000	LT598496	RBAN00000000	FMIB00000000
Genome size (bp)	7,069,771	7,058,875	7,365,767	6,823,298	6,970,905	7,074,838	7,182,425	6,683,691
G + C content (%)	72.2	72.2	72.3	71.5	72.5	72.8	73.0	73.6
No. of Contigs	34	28	1	63	40	1	20	1
Total genes	6859	6851	6354	6059	6859	6498	7080	6207
Protein coding genes	6126	6144	6062	5815	6164	6211	6344	5994
RNA genes	100	70	64	66	63	87	93	68
tRNAs	90	62	49	58	55	75	83	56

1, MS1-9^T; **2,** NGC1-4; **3,** *M. peucetia* DSM 43363^T (=JCM 12820^T); **4,** *M. avicenniae* DSM 45758^T (=JCM 31034^T); **5,** *M. endolithica* JCM 12677^T; **6,** *M. krabiensis* DSM 45844^T (=MA-2^T); **7,** *M. costi* CS1-12^T; **8**, *M. chersina* DSM 44151^T (=JCM 9459^T).
[a] Data obtained from GenBank.
[b] Data obtained from Carro et al. [4].

Table 5
ANIb, ANIm and dDDH values among the draft genomes of strains MA1-9^T and NGC1-4 and their related type strains.

Query genome	Reference genome	ANIb (%)	ANIm (%)	% dDDH (formula 2[a])	Model C.I.	Distance	Prob. DDH > = 70%	G + C difference
1	2	99.13	99.15	99.2	[91.0–94.5%]	0.009	96.69	0.01
1	3	89.28	90.48	38.7	[36.2–42.2%]	0.300	17.76	0.66
1	4	85.47	87.77	31.0	[28.6–33.5%]	0.137	0.16	0.81
1	5	83.96	87.15	28.9	[26.6–31.4%]	0.148	0.07	0.62
1	6	81.64	86.35	26.8	[24.4–29.3%]	0.162	0.02	0.12
1	7	81.57	86.21	25.9	[23.5–28.3%]	0.168	0.01	1.37
1	8	80.99	86.08	25.7	[23.4–28.8%]	0.169	0.01	0.29

Strains: **1,** MS1-9^T; **2,** NGC1-4; **3,** *M. avicenniae* DSM 45758^T (=JCM 31034^T); **4,** *M. costi* CS1-12^T; **5,** *M. krabiensis* DSM 45844^T (=MA-2^T); **6,** *M. peucetia* DSM 43363^T (=JCM 12820^T); **7**, *M. chersina* DSM 44151^T (=JCM 9459^T); **8,** *M. endolithica* JCM 12677^T. All data were obtained from this study.
[a] Recommended formula (identities/HSP length), which is liberated of genome length and is thus prosperous against the use of incomplete draft genomes.

sequence identities with putative biosynthetic terpene, lycopene cyclase, encoded in the genomes of *M. avicenniae* DSM 45758^T (BXA10_RS18475, accession no. SIR53898), *M. peucetia* DSM 43363^T (GA0070608_4935, accession no. SCL72304) and *M. krabiensis* DSM 45344^T (GA0070620_07230, accession no. WP_091589133), respectively. The terpene gene encodes in strains MS1-9^T and NGC1-4 was surrounded by other biosynthetic genes consisted of two encoding for short-chain dehydrogenase/reductase SDR (SMCOG1001)

and metallo-β-lactamase family protein (SMCOG1170), and others encoding for regulatory and transport proteins, so suggesting the presence of a biosynthetic gene cluster though the functionality and product generated by this biosynthetic cluster has still to be established. Moreover, the genomes of the both strains contained the bioclusters which showed similarities against (i) lymphostin, an immunosuppressive drug produced by *Streptomyces* sp. KYI 1783 [31] (this biosynthetic gene cluster was found in the genomes of *M. avicenniae* DSM 45758^T and *M. krabiensis* DSM 45344^T); (ii) desferrioxamine produced by *Streptomyces argillaceus* ATCC 12,956 [2] (this biocluster was also detected in the genomes of *M. avicenniae* DSM 45758^T and *M. krabiensis* DSM 45344^T); (iii) hopene, a pentacyclic terpenoid produced by *Streptomyces coelicolor* A3(2) [35], this biosynthetic gene cluster was observed in the genomes of *M. avicenniae* DSM 45758^T, *M. krabiensis* DSM 45344^T and *M. viridifaciens* DSM 43909^T; (iv) himastatin, an antitumour agent from *Streptomyces hygroscopicus* ATCC 53653^T [25] (this biocluster was also presented in the genomes of *M. avicenniae* DSM 45758^T and *M. citrea* DSM 43903^T); and (v) lipstatin, a pancreatic lipase inhibitor produced by *Streptomyces toxytricini* ATCC 19813^T [52], this biosynthetic gene cluster was also found in the genomes of *M. avicenniae* DSM 45758^T and *M. nigra* DSM 43818^T.

As mentioned above, the phenotypic, chemotaxonomic, phylogenetic and genomic data of strains MS1-9^T and NGC1-4 indicated that both are significantly different from their closely related type strains. Consequently, strains MS1-9^T and NGC1-4 represent a novel species of the genus *Micromonospora*, for which the name of *Micromonospora musae* sp. nov. is proposed.

A formal description of the novel species *Micromonospora musae* sp. nov. is shown in Table 2 with the Taxonumber TA00874.

The DDBJ accession number for the 16S rRNA gene and gyrase subunit B gene sequences of strains MS1-9^T and NGC1-4 are LC177516 and LC411966, and LC411965 and LC411967, respectively. The GenBank/EMBL/DDBJ accession numbers of the draft genome of strains MS1-9^T and NGC1-4, *Micromonospora costi* CS1-12^T and *Micromonospora endolithica* JCM 12677^T are RAZT00000000, RAZS00000000, RBAN00000000 and RBAK00000000.

Ethical approval

This article does not contain any studies with human participants and/ or animals performed by any of the authors. The formal consent is not required in this study.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.syapm.2019.126020.

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