Antonie van Leeuwenhoek (2021) 114:697–708
https://doi.org/10.1007/s10482-021-01550-6

ORIGINAL PAPER

Micromonospora rubida sp. nov., a novel actinobacterium isolated from soil of Harbin

Xiujun Sun · Shiwen Qiu · Xianxian Luo · Pinjiao Jin · Junwei Zhao · Xianyao Wu · Jize Yang · Xiangjing Wang · Jia Song · Wensheng Xiang

Received: 24 November 2020 / Accepted: 20 February 2021 / Published online: 5 March 2021
© The Author(s), under exclusive licence to Springer Nature Switzerland AG part of Springer Nature 2021

Abstract A novel actinobacterium, designated strain NEAU-HG-1^T, was isolated from soil collected from Harbin, Heilongjiang Province, Northeast China and characterised using a polyphasic approach. On the basis of 16S rRNA gene sequence analysis, strain NEAU-HG-1^T belonged to the genus *Micromonospora*, and shared high sequence similarities with *Micromonospora auratinigra* DSM 44815^T (98.9%) and *Micromonospora coerulea* DSM 43143^T (98.7%). Morphological and chemotaxonomic characteristics of the strain also supported its assignment to the genus *Micromonospora*. Cell wall contained *meso*-diaminopimelic acid and the whole-cell sugars were arabinose and xylose. The polar lipid contained diphosphatidylglycerol, phosphatidylethanolamine, glycolipid and phosphatidylinositol. The predominant menaquinones were MK-10(H_2), MK-10(H_4) and MK-10(H_6). The major fatty acids were $C_{17:0}$ cycle, *iso*-$C_{15:0}$, and *iso*-$C_{16:0}$. Furthermore, strain NEAU-HG-1^T displayed a DNA–DNA relatedness of 33.8 ± 2.2% with *M. coerulea* DSM 43143^T. The level of digital DNA–DNA hybridization between strain NEAU-HG-1^T and *M. auratinigra* DSM 44815^T was 27.2% (24.8–29.7%). The value was well below the criteria for species delineation of 70% for dDDH. Whole-genome average nucleotide identity analyses result also indicated that the isolate should be assigned to a new species under the genus *Micromonospora*. Therefore, it is concluded that strain NEAU-HG-1^T represents a novel species of the genus *Micromonospora*, for which the name *Micromonospora rubida* sp. nov. is proposed, with NEAU-HG-1^T (= CGMCC 4.7479^T = JCM 32386^T) as the type strain.

Keywords Genome · *gyrB* gene · *Micromonospora rubida* sp. nov · Polyphasic analysis · 16S rRNA gene

Abbreviations

ANI Average nucleotide identity

Xiujun Sun and Shiwen Qiu have contributed equally to this work.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10482-021-01550-6.

X. Sun · S. Qiu · X. Luo · P. Jin · J. Zhao · X. Wu · J. Yang · X. Wang · J. Song (✉) · W. Xiang (✉)
Key Laboratory of Agricultural Microbiology of Heilongjiang Province, Northeast Agricultural University, No. 59 Mucai Street, Xiangfang District, Harbin 150030, People's Republic of China
e-mail: songjia@neau.edu.cn

W. Xiang
e-mail: xiangwensheng@neau.edu.cn

W. Xiang
State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, People's Republic of China

MEGA	Molecular evolutionary genetics analysis
ISCC-NBS	Inter-society color council-national bureau of standards
TLC	Thin-layer chromatography
GC–MS	Gas chromatography–mass spectrometer
ISP	International *Streptomyces* project
BA	Bennett's agar
NA	Nutrient agar
CGMCC	China general microbiological culture collection center
DSM	Deutsche Sammlung von Mikroorganismen und Zellkulturen
dDDH	Digital DNA:DNA hybridization
DPG	Ddiphosphatidylglycerol
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
GL	Glycolipid
GY	Glucose–yeast extract medium
JCM	Japan collection of microorganisms
SSC	Saline-sodium citrate
UPL	Unknown phosphoglycolipid
PIM	Phosphatidylinositol mannosides
UL	Unidentified lipid

Introduction

The genus *Micromonospora* was described by Ørskov (1923) as a member of the family *Micromonosporaceae*. *Micromonospora* species were widely distributed in the environment, such as soil, insects, deep marine sediments and plants (Li and Hong 2016; Xiang et al. 2014; Veyisoglu et al. 2020; Kittiwongwattana et al. 2015). Members of the genus *Micromonospora* tended to feature in bioprospecting campaigns as they were a rich source of novel bioactive compounds of therapeutic value, such as aminoglycoside antibiotics (Kasai et al. 2000; Bérdy 2005; Genilloud 2012) and lupinacidins A and B with significant antitumour activity (Igarashi et al. 2007). All members of the genus produced a well-developed substrate mycelium that carried single spores either directly or on short sporophores. None of the strains formed aerial hyphae. They contained tetra- and hexa-hydrogenated menaquinones with either nine or ten isoprene units as predominant isoprenologues, *meso*-diaminopimelic and xylose. Saturated and unsaturated fatty acids, notably *iso*-$C_{15:0}$ and *iso*-$C_{16:0}$ have been discovered as the major fatty acids of the genus. Phosphatidylethanolamine is the diagnostic phospholipid (Carro et al. 2018). Currently, there were 105 validly named species (https://lpsn.dsmz.de/genus/micromonospora) including the latest described species *Micromonospora orduensis* (Veyisoglu et al. 2020), *Micromonospora deserti* (Saygin et al. 2020), *Micromonospora craterilacus* (Ay et al. 2020) and *Micromonospora pelagivivens* (Intra et al. 2020). In this study, we describe a novel species, strain NEAU-HG-1^T, isolated from soil. Here we report on the taxonomic characterization and classification of the isolate and propose that strain NEAU-HG-1^T represents a new species of the genus *Micromonospora*, for which the name *Micromonospora rubida* sp. nov. is proposed.

Materials and methods

Isolation and maintenance of the organism

Strain NEAU-HG-1^T was isolated from soil collected from Harbin, Heilongjiang Province, Northeast China (45°36′ N, 127°41′ E). The soil sample was air-dried at room temperature for 14 days before isolation. For isolation, 5 g dried soil was diluted in sterile distilled water (45 ml), and then the soil suspension was incubated in a constant temperature shaker at 28 °C and 250 g. for 30 min. Subsequently, the strain was isolated using the standard dilution plate method and grown on sodium succinate-asparagine agar (Piao et al. 2017) supplemented with cycloheximide (50 mg l^{-1}) and nalidixic acid (20 mg l^{-1}). After 3 weeks of aerobic incubation at 28 °C, colonies were transferred and purified on oatmeal agar [International *Streptomyces* Project (ISP) medium 3] (Shirling and Gottlieb 1966) and maintained as glycerol suspensions (30%, v/v) at − 80 °C. Reference type strains *M. auratinigra* DSM 44815^T, *Micromonospora olivasterospora* JCM 7348^T, *Micromonospora pisi* DSM 45175^T, *Micromonospora soli* NBRC 110009^T and *Micromonospora kangleipakensis* DSM 45612^T were purchased and isolated from peat swamp forest soil (Thawai et al. 2004), soil of Hiroshima (Kawamoto et al. 1983), root nodules of *Pisum sativum* (Garcia et al. 2010), rice rhizosphere soil (Thawai et al. 2016) and sample of limestone quarry (Nimaichand et al.

2013), respectively. However, the isolation source of strain *M. coerulea* DSM 43143^T had not been reported.

Phenotypic characterization

Morphological characteristics were observed by light microscopy (Nikon ECLIPSE E200) and scanning electron microscopy (Hitachi SU8010) using cultures grown on ISP 3 agar at 28 °C for 4 weeks (Jin et al. 2019). Samples for scanning electron microscopy were prepared by cutting a block from an agar plate and then fixing it in 2.5% glutaraldehyde buffer (pH 7.2) at 4 °C for approximately 1.5 h. After rinsing twice with phosphate buffer, samples were dehydrated through a graded series of ethanol, passed through tert-butanol and then critically point dried. The dried samples were placed onto a stub bearing adhesive and spatter-coated with gold under vacuum. (Guan et al. 2015). The cultural characteristics of strain NEAU-HG-1^T were determined after 14 days at 28 °C on various ISP (ISP 1–7) media (Shirling and Gottlieb 1966), Bennett's agar (Waksman 1967) and nutrient agar (Jones 1949). ISCC-NBS colour charts (Kelly 1964) were used to determine colours of aerial and substrate mycelia. The cell motility was identified depending on turbidity development in a tube containing semisolid medium (Leifson 1960). Gram reaction was determined by using the KOH lysis test method (Cerny 1978). Growth at different temperatures (4, 10, 15, 18, 25, 28, 32, 35, 37, 40 and 42 °C) was determined on ISP 3 agar after incubation for 14 days. The pH range for growth (pH 3–12, at intervals of 1 pH units) was tested in glucose-yeast extract broth (GY) (Jia et al. 2013) using the buffer system: pH 4.0–5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0, 0.1 M KH_2PO_4/0.1 M NaOH; pH 9.0–10.0, 0.1 M $NaHCO_3$/0.1 M Na_2CO_3; pH 11.0–12.0, 0.2 M KH_2PO_4/0.1 M NaOH (Cao et al. 2020; Zhao et al. 2019). NaCl tolerance (0–12%, with an interval of 1%, w/v) for growth were tested after 14 days growth in GY broth at 28 °C. The utilization of sole carbon and nitrogen sources, decomposition of cellulose, hydrolysis of starch and aesculin, reduction of nitrate, coagulation and peptonization of milk, liquefaction of gelatin and production of H_2S were examined as described previously (Gordon et al. 1974; Yokota et al. 1993). Hydrolysis of Tweens (20, 40 and 80) and production of urease were tested as described by Smibert and Krieg (1994). The related type strains were also included for comparison in all tests.

Chemotaxonomic analyses

For the chemotaxonomic analysis, freeze-dried biomass was prepared from cultures grown in GY medium on a rotary shaker (250 g) at 28 °C for 7 days. The isomer of diaminopimelic acid in the cell-wall hydrolysates was derivatized according to McKerrow et al. (2000) and analysed by the HPLC method described by Yu et al. (2013). The whole-cell sugars were performed according to the procedures developed by Lechevalier and Lechevalier (1980). The phospholipids in cell were separated by two-dimensional TLC and identified using the method of Minnikin et al. (1984). Menaquinones were extracted from freeze-dried biomass and purified according to Collins (1985). Extracts were analysed by a HPLC–UV method (Wu et al. 1989) using an Agilent Extend-C_{18} Column (150 × 4.6 mm, i.d. 5 μm), typically at 270 nm. The mobile phase was acetonitrile/propyl alcohol (60:40, v/v) (Song et al. 2019). The presence of mycolic acids was checked by the acid methanolysis method of Minnikin et al. (1980). To determine cellular fatty acid compositions, strain NEAU-HG-1^T and its closely related strains were cultivated in GY medium in shake flasks at 28 °C for 7 days. Fatty acid methyl esters were extracted from the biomass as described by Gao et al. (2014) and analysed by GC–MS using the method of Xiang et al. (2011).

DNA preparation, amplification and determination of 16S rRNA and *gyrB* gene sequences

Extraction of chromosomal DNA and PCR amplification of the 16S rRNA gene sequence was carried out according to the procedure developed by Kim et al. (2000). The PCR product was purified and cloned into the vector pMD19-T (Takara) and sequenced using an Applied Biosystems DNA sequencer (model 3730XL). The almost full-length 16S rRNA gene sequence of strain NEAU-HG-1^T, comprising 1509 bp, was obtained and compared with type strains available in the EzBioCloud server (https://www.ezbiocloud.net/) (Yoon et al. 2017a) and retrieved using NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi;), and then submitted to the GenBank database. PCR amplification and gene sequencing of *gyr*B

was carried out using primers GYF1 and GYR3B, as described by Garcia et al. (2010). The *gyrB* gene was proposed by Yamamoto and Harayama (1995) as a phylogenetic marker for bacterial identification and classification (Harayama and Yamamoto 1996; Yamamoto and Harayama 1996; Yamamoto et al. 1999). The *gyrB*-based methods would be more useful than 16S rDNA-based methods because the *gyrB* was a single-copy gene which encoded the ATPase domain of DNA gyrase, an enzyme essential for DNA replication (Huang 1996) in almost all of the bacteria examined (Watanabe et al. 1998, 1999). Sequences were multiple aligned in Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0 using the Clustal W algorithm and trimmed manually where was necessary. Phylogenetic trees were constructed with maximum likelihood (Felsenstein 1981) and neighbour-joining (Saitou and Nei 1987) algorithms using MEGA 7.0 (Kumar et al. 2016). The stability of the topology of the phylogenetic tree was assessed using the bootstrap method with 1000 repetitions (Felsenstein 1985). A distance matrix was generated using Kimura's two-parameter model (Kimura 1980). All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). 16S rRNA gene sequence similarities between strains were calculated on the basis of pairwise alignment using the Ezbiocloud (Yoon et al. 2017a).

Genomic analysis, DNA-DNA hybridization and DNA G + C content

The genomic DNA of strain NEAU-HG-1^T was extracted by SDS method for genome sequencing and assembly. The harvested DNA was detected by the agarose gel electrophoresis and quantified by Qubit. Whole-genome sequencing was performed on the Illumina HiSeq PE150 platform. A-tailed, ligated to paired-end adaptors and PCR amplified with a 350 bp insert was used for the library construction at the Beijing Novogene Bioinformatics Technology Co., Ltd. Illumina PCR adapter reads and low quality reads from the paired-end were filtered by the step of quality control using our own compling pipeline. All good quality paired reads were assembled using the SOAP denovo (Li et al. 2010, 2008) (http://soap.genomics.org.cn/soapdenovo.html) into a number of scaffolds. Then the filter reads were handled by the next step of the gap-closing. Whole-genome phylogeny was generated using TYGS server (http://tygs.dsmz.de) (Meier-Kolthoff and Göker 2019). "Antibiotics and secondary metabolite analysis shell" (antiSMASH) version 5.0 was employed to analyze the bioactive secondary metabolites (Blin et al. 2019). The DNA G + C content was calculated from the genome sequence.

Digital DNA-DNA hybridization (dDDH) and average nucleotide identity (ANI) values were employed to further distinguish strain NEAU-HG-1^T from its phylogenetic relatives with available genome sequences (Yoon et al. 2017b; Meier-Kolthoff et al. 2013). In the present study, ANI and dDDH values were determined from the genomes of strain NEAU-HG-1^T, *M. auratinigra* DSM 44815^T, *M. kangleipakensis* DSM 45612^T, *M. olivasterospora* JCM 7348^T and *M. pisi* DSM 45175^T using the ortho-ANIu algorithm from Ezbiocloud (Yoon et al. 2017a, b) and the genome-to-genome distance calculator (GGDC 2.1) (Meier-Kolthoff et al. 2013) at http://ggdc.dsmz.de. Because of lacking whole genome sequence of closely related strain *M. coerulea* DSM 43143^T, DNA-DNA relatedness test between them was carried out by the thermal renaturation method described by De Ley et al. (1970) under consideration of the modifications described by Huss et al. (1983), using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in-situ* temperature probe (Varian). The concentration and purity of DNA samples were determined by measuring the optical density at 260, 280 and 230 nm. The DNA samples used for hybridization were diluted to OD_{260} around 1.0 using 0.1 × SSC (saline sodium citrate buffer (Thomas et al. 2000), then sheared using a JY92-II ultrasonic cell disruptor (ultrasonic time 3 s, interval time 4 s, 90 times). The DNA renaturation rates were determined in 2 × SSC at 70 °C. The experiments were performed with three replications and the DNA–DNA relatedness value was expressed as mean of the three values.

Results and discussion

Phenotypic characteristics

Morphological observation of 4-week-old cultures of strain NEAU-HG-1^T grown on ISP 3 medium revealed that it showed morphology consistent with the genus *Micromonospora*. Strain NEAU-HG-1^T was observed to be an aerobic, Gram-stain positive actinobacterium and produced well-developed, branched and non-fragmented substrate mycelium, while the aerial mycelium was absent. Non-motile and oval spores (0.5–0.6 × 0.7–0.9 μm) developed singly on the substrate mycelium (Fig. 1). Strain NEAU-HG-1^T was found to grow well on ISP 1, ISP 3 and Bennett's agar; grow moderately on ISP 4 medium; grow poorly on ISP 2, ISP 6 and ISP 7 media; but no growth occurred on ISP 5 and nutrient agar media. The colour of the substrate hyphae was strong orange yellow to vivid reddish orange, but no diffusible pigment was observed on any of the media tested for the isolate. The summaries of cultural characteristics of strain NEAU-HG-1^T were shown in Table S1. The strain was found to grow at a temperature range of 10–37 °C (optimum temperature 28 °C), pH 6–12 (optimum pH of 7) and in the presence of NaCl up to 3% (w/v). The physiological and biochemical properties of strain NEAU-HG-1^T were shown in Table 1.

Chemotaxonomic characteristics

All the chemotaxonomic data were consistent with the assignment of strain NEAU-HG-1^T to the genus *Micromonospora*. The isolate was determined to contain *meso*-diaminopimelic acid in the cell wall. Whole-cell sugars contained arabinose and xylose. The phospholipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, glycolipid and phosphatidylinositol (Fig. S1). The menaquinones were MK-10(H_6) (45.9%), MK-10(H_4) (33.6%), MK-10(H_2) (10.1%), MK-10(H_8) (6.8%), MK-9(H_4) (1.7%), MK-9(H_6) (1.3%) and MK-9(H_2) (0.6%). The cellular fatty acid profile was composed of $C_{17:0}$ cycle (25.7%), *iso*-$C_{15:0}$ (18.8%), *iso*-$C_{16:0}$ (10.5%), $C_{17:0}$ (7.9%), $C_{18:1}$ (7.4%), *iso*-$C_{18:0}$ (7.3%), 10-methyl $C_{17:0}$ (6.9%), *iso*-$C_{17:0}$ (4.4%), $C_{19:1}$ (3.3%), $C_{18:0}$ (2.4%), *iso*-$C_{16:1}$ (1.4%), 10-methyl $C_{18:0}$ (1.3%), *anteiso*-$C_{17:0}$ (1.2%), *iso*-$C_{17:1}$ (0.9%) and *anteiso*-$C_{15:0}$ (0.6%). Mycolic acids were not found.

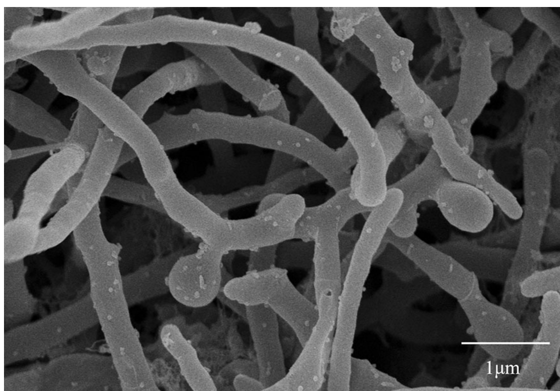


Fig. 1 Scanning electron microscopy of strain NEAU-HG-1^T grown on ISP 3 for 4 weeks at 28 °C. Bar, 1.0 μm

Molecular characteristics

EzBioCloud analysis based on 16S rRNA gene sequence (1509 bp; GenBank/EMBL/DDBJ accession number MG753996) indicated that strain NEAU-HG-1^T should be affiliated to the genus *Micromonospora* and possessed high 16S rRNA gene sequence similarities to *M. auratinigra* DSM 44815^T (98.9%) and *M. coerulea* DSM 43143^T (98.7%). Phylogenetic analysis using the 16S rRNA gene sequences showed that the strain formed a stable clade with *M. coerulea* DSM 43143^T and clustered with *M. soli* NBRC 110009^T (98.1%) and *M. kangleipakensis* DSM 45612^T (97.9%) in the neighbour-joining tree (Fig. 2). This relationship was also observed in the maximum-likelihood tree (Fig. S2). However, whole-genome phylogeny showed strain NEAU-HG-1^T formed a stable phyletic line with *M. olivasterospora* JCM 7348^T (98.1%) (Figure. S3). The partial *gyrB* gene sequence obtained for strain NEAU-HG-1^T (1161 nt) has been deposited in the GenBank/EMBL/DDBJ databases as MW091032. The *gyrB* sequence analysis showed that strain NEAU-HG-1^T was placed in the genus *Micromonospora*. Meanwhile, the phylogenetic analyses based on *gyrB* gene sequences showed that the strain NEAU-HG-1^T formed a stable clade with *M. pisi* DSM 45175^T (97.8%) in the neighbour-joining tree (Fig. S4), and the relationship was also supported by the corresponding maximum-likelihood (Fig. S5). Based on the 16S rRNA gene sequences similarities and phylogenic analysis, *M. auratinigra* DSM 44815^T, *M. coerulea* DSM

Table 1 Differential characteristics of strain NEAU-HG-1^T and its closely related species of the genus *Micromonospora*

Characteristics	1	2	3	4[b]	5[c]	6[d]	7[e]
Growth at/with:							
Temperature range (°C)	10–37	10–37	28–37	28–38	20–37	20–45	15–37
pH range	6–12	6–9	6–8	6.8–7.8	7–9	5–10	5–7
NaCl % (w/v) tolerance	0–3	0–2	0–4	0–3	0–2	0–4	0
Coagulation and peptonization of milk	−	W	−	+	ND	+	ND
Decomposition of Cellulose	−	−	−	W	ND	W	ND
Hydrolysis of starch	−	+	−	+	+	+	−
production of H_2S	−	W	−	ND	ND	−	ND
Liquefaction of gelatin	−	+	−	W	+	+	−
Hydrolysis of aesculin	W	+	W	ND	+	ND	−
Utilize as sole carbon source							
D-fructose	+	−	−	+	+	−	−
Inositol	−	−	+	−	ND	−	−
Lactose	+	+	+	−	ND	ND	−
D-mannitol	+	−	−	−	ND	−	−
D-raffinose	+	+	+	−	−	−	−
L-rhamnose	+	−	−	−	+	−	−
D-xylose	−	+	−	+	+	−	−
L-arabinose	−	+	−	−	+	+	−
D-ribose	−	+	−	+	ND	−	ND
Utilize as sole nitrogen source							
L-alanine	+	+	W	−	+	ND	+
Creatine	+	W	−	−	ND	−	−
L-glutamic acid	−	W	W	+	ND	ND	ND
L-glutamine	−	+	+	ND	ND	ND	+
L-threonine	−	+	+	+	+	ND	−
L-tyrosine	−	W	W	−	−	ND	ND
L-proline	−	+	+	ND	−	ND	−
L-serine	+	+	W	+	−	ND	+
Glycine	−	W	+	ND	ND	ND	−
Whole-cell sugars	Arabinose, Xylose	Arabinose, Xylose[a]	ND	Arabinose, Xylose, Glucose	Galactose, Xylose, Mannose, Ribose	Arabinose, Glucose, Xylose, Ribose Mannose	Galactose, Glucose, Mannose, Xylose, Rhamnose, Ribose

Table 1 continued

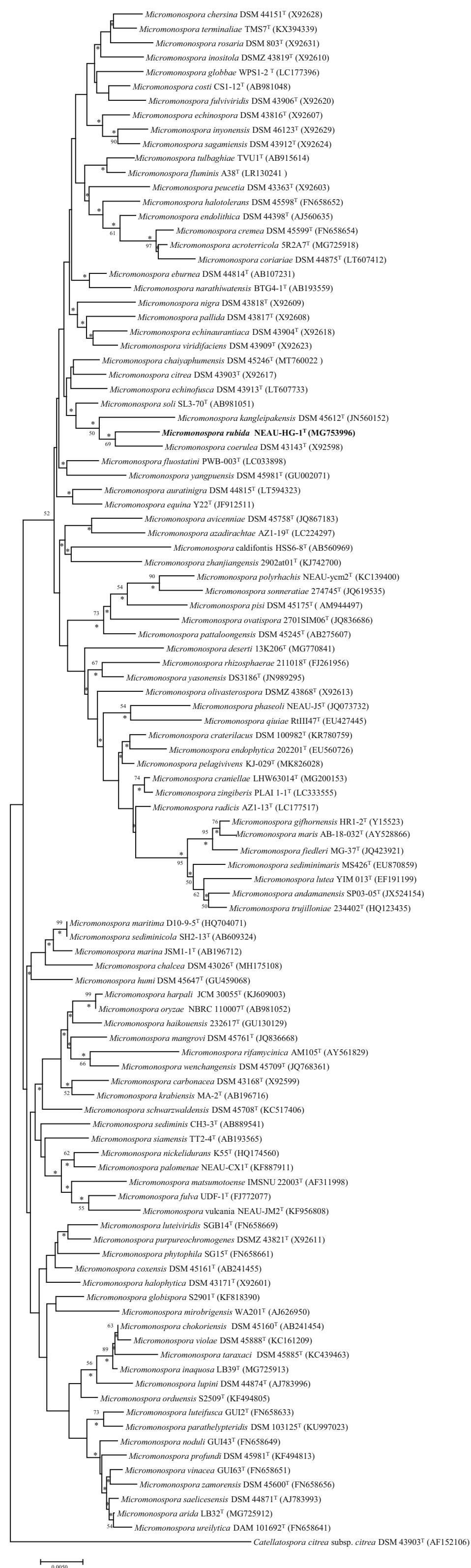
Characteristics	1	2	3	4[b]	5[c]	6[d]	7[e]
Polar lipids	DPG, PE GL, PI	DPG, PE PI, PIM[a]	ND	DPG, PE PI, PIM	DPG, PE, PG, PI, UPL	DPG, PE, PG, PI, PIM, UL, UPL	DPG, PE, PG, PI, PIM, UL

All the data from this study were obtained under the same conditions except where marked. Strains: 1, NEAU-HG-1^T; 2, *M. auratinigra* DSM 44815^T; 3. *M. coerulea* DSM 43143^T; 4. *M. olivasterospora* JCM 7348^T; 5. *M. pisi* DSM 45175^T; 6. *M. soli* NBRC 110009^T 7. *M. kangleipakensis* DSM 45612^T. +, positive; −, negative; ND, no data available; W, weakly positive. [a]Data from Thawai et al. (2004); [b]Data from Kawamoto et al. (1983); [c]Data from Garcia et al. (2010); [d] Date from Thawai et al. (2016); [e] Data from Nimaichand et al. (2013)

43143^T, *M. olivasterospora* JCM 7348^T, *M. pisi* DSM 45175^T, *M. soli* NBRC 110009^T and *M. kangleipakensis* DSM 45612^T were selected as the reference strains for comparative analysis.

DNA-DNA hybridization by the thermal renaturation method was carried out between strain NEAU-HG-1^T and *M. coerulea* DSM 43143^T to determine whether the isolate represented a novel species. Strain NEAU-HG-1^T showed a DNA-DNA relatedness of 33.8 ± 2.2% with *M. coerulea* DSM 43143^T. Digital DNA-DNA hybridization and ANI values were employed to further clarify the relatedness between strain NEAU-HG-1^T and *M. auratinigra* DSM 44815^T, *M. kangleipakensis* DSM 45612^T, *M. olivasterospora* JCM 7348^T and *M. pisi* DSM 45175^T. The levels of digital DNA-DNA hybridization between them were 27.2% (24.8–29.7%), 29.2% (26.8–31.7%), 32.4% (29.9–34.9%) and 22.5% (20.2–24.9%), respectively. These values were below the threshold value of 70% recommended by Wayne et al. (1987) for assigning strains to the same genomic species. Similarly, the low ANI values between strain NEAU-HG-1^T and its reference strains *M. auratinigra* DSM 44815^T, *M. kangleipakensis* DSM 45612^T, *M. olivasterospora* JCM 7348^T and *M. pisi* DSM 45175^T were found to be 83.7%, 84.8%, 86.4% and 78.6%, respectively, a result well below the threshold used to delineate prokaryote species (Richter and Rossello-Mora 2009; Chun and Rainey 2014).

The assembled genome sequence of strain NEAU-HG-1^T was found to be 7,473,743 bp long and composed of 37 scaffolds with an N50 of 463,225 bp, a DNA G + C content of 72.8% and a coverage of 236 X. It was deposited in GenBank database under the accession number JAAALO000000000. NCBI Prokaryotic Genome Annotation Pipeline (PGAP) revealed four copies of the 5S rRNA genes, one copy of the 16S rRNA gene, one copy of the 23S rRNA gene, 51 tRNA genes, three copies of noncoding RNA genes and 6067 protein-coding genes (CDSs). Detailed genomic information and other general features of genome sequences were shown in Table S2. Among these CDSs, 5133 (84.6%) genes were classified into 24 clusters of orthologous groups of proteins. Most of the genes were associated with functions such as transcription (559), carbohydrate transport and metabolism (376), amino acid transport and metabolism (371), signal transduction mechanisms (309), lipid transport and metabolism (265), coenzyme transport and metabolism (264), cell wall/membrane/envelope biogenesis (263), translation, ribosomal structure and biogenesis (262), inorganic ion transport and metabolism (257), energy production and conversion (256), and secondary metabolites biosynthesis, transport and catabolism (227). These functions were essentials for nutritional/spatial competition and antagonism against microorganisms to compete in various ecosystems (Liu et al. 2019). Genome mining analysis using antiSMASH 5.0 led to the identification of 24 putative gene clusters in the genome of strain NEAU-HG-1^T, including 20 gene clusters that showed very low similarity to the known gene clusters of hopene, kirromycin, nystatin-like Pseudonocardia polyene, divergolide A, nostopeptolide A2, enduracidin, formicamycins A-M, gentamicin and so on. Hence, it had great potential to biosynthesize various secondary metabolite and produce novel bioactive compounds. One cluster showed 83% similarity to the reported biosynthetic gene cluster of desferrioxamine B in *Streptomyces*



◀**Fig. 2** Neighbour-joining tree showing the phylogenetic position of strain NEAU-HG-1^T (1509 bp) and the 105 phylogenetically closely related representative species with validly-published names in the genus *Micromonospora*. The out-group used was *Catellatospora citrea* subsp. *citrea* strain DSM 43903^T. Only bootstrap values above 50% (percentages of 1000 replications) are indicated. *Asterisks* indicate branches also recovered in the maximum-likelihood tree; Bar, 0.005 nucleotide substitutions per site

coelicolor A3(2) (Becerril et al. 2018). The other three putative gene clusters with more than 50% similarity to the known clusters were related to the production of catenulipeptin (Wang and van der Donk 2012), alkyl-*O*-dihydrogeranyl-methoxyhydroquinones (Awakawa et al. 2011) and avilamycin A (Weitnauer et al. 2001). The strain NEAU-HG-1^T contained genes predicted to code for indole-3-glycerol phosphate synthase, an intermediate in the tryptophan synthetic pathway associated with the production of indol-acetic acid (IAA) which stimulated plant growth (Ouyang et al. 2000). Genome analysis also showed that strain NEAU-HG-1^T contained 115 glycoside hydrolases (GHs), 103 glycosyl transferases (GTs), 15 carbohydrate esterases (CEs), 5 auxiliary activities (AAs) and 2 polysaccharide lyases (PLs) genes. To sum up, it was speculated that strain NEAU-HG-1^T had immense potential to be a rich source for producing various bioactive compounds and display striking functions.

Beside of the genotypic evidence above, the strain NEAU-HG-1^T also could differentiate from its closely related strains by phenotypic characteristics (Table 1). Differential cultural characteristics contained: pH range of strain NEAU-HG-1^T was up to 12, which is higher than that of other reference strains. Other phenotypic differences included: temperature range and NaCl tolerance for growth, hydrolysis of starch and aesculin, decomposition of cellulose, liquefaction of gelatin, production of H_2S, coagulation and peptonization of milk and utilization of inositol, D-mannitol, D-raffinose, D-fructose, lactose, L-rhamnose, D-xylose, L-arabinose, D-ribose, L-alanine, creatine, L-glutamic acid, L-glutamine, L-threonine, L-tyrosine, glycine, L-proline and L-serine. Therefore, based on a combination of chemotaxonomic, morphological, molecular and physiological data, strain NEAU-HG-1^T represents a novel species of the genus *Micromonospora*, for which the name *Micromonospora rubida* sp. nov. is proposed.

Description of *Micromonospora rubida* sp. nov.

Micromonospora rubida (ru'bi.da. L. fem. adj. rubida reddish)

Aerobic, Gram-stain-positive actinobacterium that forms single oval spores on well-developed branched substrate hyphae. Aerial mycelium is not produced and the spores are 0.5–0.6 × 0.7–0.9 µm in size. Good growth on ISP 1, ISP 3 and Bennett's agar media; moderate growth on ISP 4 medium; poor growth on ISP 2, ISP 6 and ISP 7 media; but no growth on ISP 5 and nutrient agar media. The colour of the substrate hyphae is strong orange yellow to vivid reddish orange, and no soluble pigment is produced on all used media. The growth temperature range is 10–37 °C, with an optimum at 28 °C. Growth is occurred at pH 6–12 (optimum pH 7.0). The maximum NaCl concentration for growth is 3.0% (w/v). Weakly positive for hydrolysis of aesculin, but negative for hydrolysis of Tweens (20,40 and 80) and starch, liquefaction of gelatin, production of urease, coagulation and peptonization of milk, decomposition of cellulose, production of H_2S, and reduction of nitrate. Utilize raffinose, D-mannitol, D-galactose, D-glucose, lactose, maltose, L-rhamnose, D-fructose and sucrose as the sole carbon source for growth, while the following substrates are not used as carbon sources: inositol, L-arabinose, D-mannose, D-ribose, D-sorbitol and D-xylose. L-alanine, L-arginine, L-aspartic acid, L-asparagine, L-serine and creatine are utilized as the sole nitrogen source, but not L-glutamic acid, L-glutamine, L-proline, L-threonine, L-tyrosine or glycine. Cell walls contain *meso*-diaminopimelic acid as the diagnostic diamino acid and the whole-cell sugars are arabinose and xylose. The phospholipid profile is identified to contain diphosphatidylglycerol, phosphatidylethanolamine, glycolipid and phosphatidylinositol. The predominant menaquinones (> 10%) are MK-10(H_2), MK-10(H_4) and MK-10(H_6). The major fatty acids (> 10%) are $C_{17:0}$ cycle, *iso*-$C_{15:0}$, and *iso*-$C_{16:0}$. The DNA G + C content of the type strain is 72.8%.

The type strain is NEAU-HG-1^T (= CGMCC 4.7479^T = JCM 32386^T), isolated from soil collected from Harbin, Heilongjiang Province, Northeast China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NEAU-HG-1^T is MG753996. The GenBank/EMBL/DDBJ accession number for the partial *gyrB* gene sequence of strain NEAU-HG-1^T is MW091032. The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAAALO000000000. The version described in this paper is version JAAALO000000000.1.

Acknowledgements We thank Prof. Aharon Oren for his valuable help with naming the species.

Author's contribution Xiujun Sun and Shiwen Qiu performed the laboratory experiments, analysed the data, and drafted the manuscript. Xianxian Luo contributed to the biochemical characterization. Pinjiao Jin contributed to the polyphasic taxonomy. Junwei Zhao contributed to the fatty acids determination. Xianyao Wu and Jize Yang contributed to the morphological analyses. Xiangjing Wang participated to the discussions of experiments and revised the manuscript. Jia Song and Wensheng Xiang designed the experiments and revised the manuscript.

Funding This work was supported in part by grants from the National Natural Science Foundation of China (No. 31700067), the China Postdoctoral Science Foundation (2015M580255), the Heilongjiang Provincial Postdoctoral Science Foundation (LBH-Z15016), and the "Academic Backbone" Project of Northeast Agricultural University (19XG18).

Data availability The GenBank/EMBL/DDBJ Accession Number for the 16S rRNA gene sequence of strain NEAU-HG-1^T is MG753996. The GenBank/EMBL/DDBJ accession number for the partial *gyrB* gene sequence of strain NEAU-HG-1^T is MW091032. The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAAALO000000000. The version described in this paper is version JAAALO000000000.1.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethics 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.

Consent to participate and/or consent to publish This research doesn't involve in human subjects, so the informed consent to participate and consent to publish are not obtained.

Informed consent All authors have seen a copy of the manuscript and have approved its submission.

References

Awakawa T, Fujita N, Hayakawa M, Ohnishi Y, Horinouchi S (2011) Characterization of the biosynthesis gene cluster for alkyl-*O*-dihydrogeranyl-methoxyhydroquinones in *Actinoplanes missouriensis*. ChemBioChem 12:439–448

Ay H, Nouioui I, Klenk HP, Cetin D, Igual JM, Sahin N, Isik K (2020) Genome-based classification of *Micromonospora craterilacus* sp. nov., a novel actinobacterium isolated from Nemrut Lake. Antonie Van Leeuwenhoek 113:791–801

Becerril A, Álvarez S, Braña AF, Rico S, Díaz M, Santamaría RI, Salas JA, Méndez C (2018) Uncovering production of specialized metabolites by *Streptomyces argillaceus*: Activation of cryptic biosynthesis gene clusters using nutritional and genetic approaches. PLoS ONE 13:e0198145

Bérdy J (2005) Bioactive microbial metabolites. J Antibiot 58:1–26

Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, Medema MH, Weber T (2019) antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Res 47:W81–W87

Cao P, Li CX, Tan KF, Liu CZ, Xu X, Zhang SY, Wang XJ, Zhao JW, Xiang WS (2020) Characterization, phylogenetic analyses and pathogenicity of *Enterobacter cloacae* on rice seedlings in Heilongjiang Province, China. Plant Dis. https://doi.org/10.1094/PDIS-12-19-2557-RE

Carro L, Nouioui I, Sangal V, Meier-Kolthoff JP, Trujillo ME, Montero-Calasanz MDC, Sahin N, Smith DL, Kim KE, Peluso P, Deshpande S, Woyke T, Shapiro N, Kyrpides NC, Klenk HP, Göker M, Goodfellow M (2018) Genome-based classification of micromonosporae with a focus on their biotechnological and ecological potential. Sci Rep 8:525

Cerny G (1978) Studies on the aminopeptidase test for the distinction of gram-negative from gram-positive bacteria. Eur J Appl Microbiol Biotechnol 5:113–122

Chun J, Rainey FA (2014) Integrating genomics into the taxonomy and systematics of the Bacteria and Archaea. Int J Syst Evol Microbiol 64:316–324

Collins MD (1985) Chemical methods in bacterial systematics. In: Goodfellow M, Minnikin DE (eds) Isoprenoid quinone analyses in bacterial classification and identification. Academic Press, London, pp 267–284

De Ley J, Cattoir H, Reynaerts A (1970) The quantitative measurement of DNA hybridization from renaturation rates. Eur J Biochem 12:133–142

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Gao RX, Liu CX, Zhao JW, Jia FY, Yu C, Yang LY, Wang XJ, Xiang WS (2014) *Micromonospora jinlongensis* sp. nov., isolated from muddy soil in China and emended description of the genus *Micromonospora*. Antonie Van Leeuwenhoek 105:307–315

Garcia LC, Martínez-Molina E, Trujillo ME (2010) *Micromonospora pisi* sp. nov., isolated from root nodules of *Pisum sativum*. Int J Syst Evol Microbiol 60:331–337

Genilloud O (2012) Genus I. *Micromonospora* Ørskov 1923, 156AL. In Bergey's Manual of Systematic Bacteriology, 2nd edn.vol. 5 The *Actinobacteria*, Part B pp.1039–1057 Edited by Goodfellow M, Kämpfer P, Busse HJ, Trujillo ME, Suzuki KI, Ludwig W, Whitman WB. New York: Springer

Gordon RE, Barnett DA, Handerhan JE, Pang C (1974) *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. Int J Syst Bacteriol 24:54–63

Guan XJ, Liu CX, Zhao JW, Fang BZ, Zhang YJ, Li LJ, Jin PJ, Wang XJ, Xiang WS (2015) *Streptomyces maoxianensi*s sp. nov., a novel actinomycete isolated from soil in Maoxian, China. Antonie Van Leeuwenhoek 107:1119–1126

Harayama S, Yamamoto S (1996) Phylogenetic identification of *Pseudomonas* strains based on a comparison of *gyrB* and *rpoD* sequences. In: Nakazawa T, Furukawa K, Haas D, Silver S (eds) Molecular biology of pseudomonads. ASM Press, Washington, pp 250–258

Huang WM (1996) Bacterial diversity based on type II DNA topoisomerase genes. Annu Rev Genet 30:79–107

Huss VAR, Festl H, Schleifer KH (1983) Studies on the spectrometric determination of DNA hybridization from renaturation rates. Syst Appl Microbiol 4:184–192

Igarashi Y, Trujillo ME, Martínez-Molina E, Yanase S, Miyanaga S, Obata T, Sakurai H, Saiki I, Fujita T, Furumai T (2007) Antitumor anthraquinones from an endophytic actinomycete *Micromonospora lupini* sp. nov. Bioorg Med Chem Lett 17:3702–3705

Intra B, Panbangred W, Inahashi Y, Také A, Mori M, Ōmura S, Matsumoto A (2020) *Micromonospora pelagivivens* sp. nov., a new species of the genus *Micromonospora* isolated from deep-sea sediment in Japan. Int J Syst Evol Microbiol 70:3069–3075

Jia FY, Liu CX, Wang XJ, Zhao JW, Zhang LQF, J, Gao RX, Xiang WS, (2013) *Wangella harbinensis* gen. nov., sp. nov., a new member of the family *Micromonosporaceae*. Antonie Van Leeuwenhoek 103:399–408

Jin LY, Zhao Y, Song W, Duan LP, Jiang SW, Wang XJ, Zhao JW, Xiang WS (2019) *Streptomyces inhibens* sp. nov., a novel actinomycete isolated from rhizosphere soil of wheat (*Triticum aestivum* L.). Int J Syst Evol Microbiol 69:688–695

Jones KL (1949) Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. J Bacteriol 57:141–145

Kasai H, Tamura T, Harayama S (2000) Intrageneric relationships among *Micromonospora* species deduced from *gyr*B-based phylogeny and DNA relatedness. Int J Syst Evol Microbiol 50:127–134

Kawamoto I, Yamamoto M, Nara T (1983) *Micromonospora olivasterospora* sp. nov. Int J Syst Bacteriol 33:107–112

Kelly KL (1964) Inter-society colour council-national bureau of standards colour-name charts illustrated with centroid colours. US Government Printing Office, Washington

Kim SB, Brown R, Oldfield C, Gilbert SC, Iliarionov S, Goodfellow M (2000) *Gordonia amicalis* sp. nov., a novel dibenzothiophene-desulphurizing actinomycete. Int J Syst Evol Microbiol 50:2031–2036

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Kittiwongwattana C, Thanaboripat D, Laosinwattana C, Koohakan P, Parinthawong N, Thawai C (2015) *Micromonospora oryzae* sp. nov., isolated from roots of upland rice. Int J Syst Evol Microbiol 65:3818–3823

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Lechevalier MP, Lechevalier HA (1980) The chemotaxonomy of actinomycetes. In: Dietz A, Thayer DW (eds) Actinomycete taxonomy special publication, vol 6. Society of Industrial Microbiology, Arlington, pp 227–291

Leifson E (1960) Atlas of bacterial flagellation. Q Rev Biol 242

Li L, Hong K (2016) *Micromonospora ovatispora* sp. nov. isolated from mangrove soil. Int J Syst Evol Microbiol 66:889–893

Li R, Li Y, Kristiansen K, Wang J (2008) SOAP: short oligonucleotide alignment program. Bioinformatics 24:713–714

Li R, Zhu H, Ruan J, Qian W, Fang X, Shi Z, Li Y, Li S, Shan G, Kristiansen K, Li S, Yang H, Wang J, Wang J (2010) De novo assembly of human genomes with massively parallel short read sequencing. Genome Res 20:265–272

Liu D, Yan R, Fu Y, Wang X, Zhang J, Xiang W (2019) Antifungal, plant growth-promoting, and genomic properties of an endophytic actinobacterium *Streptomyces* sp. NEAU-S7GS2. Front Microbiol 10:2077

McKerrow J, Vagg S, McKinney T, Seviour EM, Maszenan AM, Brooks P, Se-viour RJ (2000) A simple HPLC method for analysing diaminopimelic acid diastereomers in cell walls of gram-positive bacteria. Lett Appl Microbiol 30:178–182

Meier-Kolthoff JP, Göker M (2019) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:2182

Meier-Kolthoff JP, Auch AF, Klenk HP, Goker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinf 14:60

Minnikin DE, Hutchinson IG, Caldicott AB, Goodfellow M (1980) Thin-layer chromatography of methanolysates of mycolic acid-containing bacteria. J Chromatogr 188:221–233

Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal K, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241

Nimaichand S, Zhang YG, Cheng J, Li L, Zhang DF, Zhou EM, Dong L, Ningthoujam DS, Li WJ (2013) *Micromonospora kangleipakensis* sp. nov., isolated from a sample of limestone quarry. Int J Syst Evol Microbiol 63:4546–4551

Ørskov J (1923) Investigations into the morphology of the ray fungi. Levin and Munksgaard, Copenhagen

Ouyang J, Shao X, Li J (2000) Indole-3-glycerol phosphate, a branchpoint of indole-3-acetic acid biosynthesis from the tryptophan biosynthetic pathway in *Arabidopsis thaliana*. Plant J 24:327–333

Piao CY, Zheng WW, Li Y, Liu CX, Jin L, Song W, Yan K, Wang XJ, Xiang WS (2017) Two new species of the genus *Streptomyces*: *Streptomyces camponoti* sp. nov. and *Streptomyces cuticulae* sp. nov. isolated from the cuticle of *Camponotus japonicus* Mayr. Arch Microbiol 199:963–970

Richter M, Rossello-Mora R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci USA 106:19126–19131

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Saygin H, Ay H, Guven K, Cetin D, Sahin N (2020) *Micromonospora deserti* sp. nov., isolated from the Karakum Desert. Int J Syst Evol Microbiol 70:282–291

Shirling EB, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol 16:313–340

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, pp 607–654

Song J, Qiu SW, Zhao JW, Han CY, Ying W, Sun XJ, Jiang SW, Wang XJ, Xiang WS (2019) *Pseudonocardia tritici* sp. nov., a novel actinomycete isolated from rhizosphere soil of wheat (*Triticum aestivum* L.). Antonie Van Leeuwenhoek 112:765–773

Thawai C, Tanasupawat S, Itoh T, Suwanborirux K, Kudo T (2004) *Micromonospora aurantionigra* sp. nov., isolated from a peat swamp forest in Thailand. Actinomycetologica 18:8–14

Thawai C, Kittiwongwattana C, Thanaboripat D, Laosinwattana C, Koohakan P, Parinthawong N (2016) *Micromonospora soli* sp. nov., isolated from rice rhizosphere soil. Antonie Van Leeuwenhoek 109:449–456

Thomas EA, Alvarez CE, Sutcliffe JG (2000) Evolutionarily distinct classes of S27 ribosomal proteins with differential mRNA expression in rat hypothalamus. J Neurochem 74:2259–2267

Veyisoglu A, Carro L, Cetin D, Igual JM, Klenk HP, Sahin N (2020) *Micromonospora orduensis* sp. nov., isolated from deep marine sediment. Antonie Van Leeuwenhoek 113:397–405

Waksman SA (1967) The actinomycetes. A summary of current knowledge. Ronald Press, New York

Wang H, van der Donk WA (2012) Biosynthesis of the class III lantipeptide catenulipeptin. ACS Chem Biol 7:1529–1535

Watanabe K, Yamamoto S, Hino S, Harayama S (1998) Population dynamics of phenol-degrading bacteria in activated sludge determined by *gyrB*-targeted quantitative PCR. Appl Environ Microbiol 64:1203–1209

Watanabe K, Teramoto M, Harayama S (1999) An outbreak of nonflocculating catabolic populations caused the breakdown of a phenol-digesting activated-sludge process. Appl Environ Microbiol 65:2813–2819

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE (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

Weitnauer G, Mühlenweg A, Trefzer A, Hoffmeister D, Süssmuth RD, Jung G, Welzel K, Vente A, Girreser U, Bechthold A (2001) Biosynthesis of the orthosomycin

antibiotic avilamycin A: deductions from the molecular analysis of the avi biosynthetic gene cluster of *Streptomyces viridochromogenes* Tü57 and production of new antibiotics. Chem Biol 8:569–581

Wu C, Lu X, Qin M, Wang Y, Ruan J (1989) Analysis of menaquinone compound in microbial cells by HPLC. Microbiology 16:176–178

Xiang WS, Liu CX, Wang XJ, Du J, Xi LJ, Huang Y (2011) *Actinoalloteichus nanshanensis* sp. nov., isolated from the rhizosphere of a fig tree (*Ficus religiosa*). Int J Syst Evol Microbiol 61:1165–1169

Xiang WS, Yu C, Liu CX, Zhao JW, Yang LY, Xie BJ, Li L, Hong K, Wang XJ (2014) *Micromonospora polyrhachis* sp. nov., an actinomycete isolated from edible Chinese black ant (*Polyrhachis vicina* Roger). Int J Syst Evol Microbiol 64:495–500

Yamamoto S, Harayama S (1995) PCR amplification and direct sequencing of *gyrB* genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains. Appl Environ Microbiol 61:1104–1109

Yamamoto S, Harayama S (1996) Phylogenetic analysis of *Acinetobacter* strains based on the nucleotide sequences of *gyrB* genes and on the amino acid sequences of their products. Int J Syst Bacteriol 46:506–511

Yamamoto S, Bouvet PJ, Harayama S (1999) Phylogenetic structures of the genus *Acinetobacter* based on *gyrB* sequences: comparison with the grouping by DNA-DNA hybridization. Int J Syst Bacteriol 49:87–95

Yokota A, Tamura T, Hasegawa T, Huang LH (1993) *Catenuloplanes japonicas* gen. nov., sp. nov., nom. rev., a new genus of the order Actinomycetales. Int J Syst Bacteriol 43:805–812

Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017a) Introducing EzBioCloud: A taxonomically united database of 16S rRNA and whole genome assemblies. Int J Syst Evol Microbiol 67:1613–1617

Yoon SH, Ha SM, Lim J, Kwon S, Chun J (2017b) A large scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110:1281–1286

Yu C, Liu CX, Wang XJ, Zhao JW, Yang LY, Gao RX, Zhang YJ, Xiang WS (2013) *Streptomyces polyrhachii* sp. nov., a novel actinomycete isolated from an edible Chinese black ant (*Polyrhachis vicina* Roger). Antonie Van Leeuwenhoek 104:1013–1019

Zhao JW, Han LY, Yu MY, Cao P, Li DM, Guo XW, Liu YQ, Wang XJ, Xiang WS (2019) Characterization of *Streptomycessporangiiformans* sp. Nov., a Novel Soil Actinomycete with Antibacterial Activity against *Ralstonia solanacearum*. Microorganisms 7:360

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.