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Streptomyces monticola sp. nov., a novel actinomycete isolated from soil

Dongmei Li · Liyuan Han · Junwei Zhao · Hanxun Ju · Shanwen Jiang · Xiaowei Guo · Xiangjing Wang · Wensheng Xiang

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Abstract A novel actinobacterium, designated strain NEAU-GS4^T, was isolated from soil collected from Mount Song and characterised using a polyphasic approach. 16S rRNA gene sequence similarity studies showed that strain NEAU-GS4^T belongs to the genus *Streptomyces*, being closely related to *Streptomyces spectabilis* JCM 4308^T (98.8%), *Streptomyces sclerotialus* DSM 43032^T (98.3%) and *Streptomyces lasiicapitis* 3H-HV17(2)^T (98.0%). A multilocus sequence analysis based on five house-keeping genes (*atp*D, *gyr*B, *rpo*B, *rec*A and *trp*B) also indicated that strain NEAU-GS4^T should be assigned to the genus *Streptomyces*. The major menaquinones were identified as MK-9(H_6), MK-9(H_8) and MK-9(H_4). The polar lipid profile was found to contain diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylinositolmannosides and an unidentified phospholipid. The major fatty acids were identified as anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$. Moreover, DNA–DNA hybridization results and some phenotypic characteristics indicated that strain NEAU-GS4^T can be clearly differentiated from its closely related species of the genus *Streptomyces*. Therefore, it is concluded that strain NEAU-GS4^T represents a novel species of the genus of *Streptomyces*, for which the name *Streptomyces monticola* sp. nov. is proposed. The type stain is NEAU-GS4^T (=CGMCC 4.7467^T = DSM 105116^T).

Keywords *Streptomyces monticola* sp. nov. · Polyphasic taxonomy · 16S rRNA gene

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D. Li · L. Han · J. Zhao · H. Ju · S. Jiang ·
X. Guo · X. Wang (✉) · W. Xiang (✉)
Key Laboratory of Agriculture Biological Functional Gene of Heilongjiang Provincial Education Committee, Northeast Agricultural University, No. 59 Mucai Street, Xiangfang District, Harbin 150030, People's Republic of China
e-mail: wangneau2013@163.com

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

Introduction

The genus *Streptomyces* was first described by Waksman and Henrici (1943) and currently consists of more than 800 species with validly published names (http://www.bacterio.net/streptomyces.html). As the largest genus of the phylum *Actinobacteria*, members of the genus *Streptomyces* are widely distributed in soils throughout the world and have a wide range of

metabolic abilities and potential applications in the production of antibiotics, enzymes, enzyme inhibitors, vitamins and bioactive compounds with importance in the food, agriculture and pharmaceutical industries (Labeda et al. 2012; Bérdy 2005). Therefore, members of novel *Streptomyces* species are in demand as a source of novel, commercially significant, naturally bioactive compounds (Berdy 1995; Fiedler et al. 2005). Our laboratory has been interested in isolating actinomycetes from soils, plants, sediments, insects and so on, with the purpose of selecting strains with potential for biotechnological applications. During our investigations into the diversity and bioactivity of actinomycetes in a soil sample, which was collected from Mount Song, a novel actinomycete strain, designated NEAU-GS4^T, was isolated. The present polyphasic taxonomic characterisation indicates that this strain represents a novel species of the genus *Streptomyces.*

Materials and methods

Isolation and maintenance of the organism

Strain NEAU-GS4^T was isolated from soil collected from Mount Song, Dengfeng, Henan Province, China (34°29′N, 113°2′E). The soil sample was air-dried at room temperature for 14 days before isolation of actinomycetes. After drying, 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 21 days of aerobic incubation at 28 °C, colonies were transferred and purified on International Streptomyces Project (ISP) medium 3 (Shirling and Gottlieb 1966) and maintained as glycerol suspensions (20%, v/v) at − 80 °C. The type strains of *Streptomyces spectabilis* JCM 4308^T and *Streptomyces sclerotialus* DSM 43032^T were purchased from the Japan Collection of Microorganisms (JCM) and Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), respectively; the strain *Streptomyces lasiicapitis* 3H-HV17(2)^T was isolated and stored in our laboratory. All strains were cultured under the same conditions for comparative analysis.

Morphological, cultural and physiological characteristics

Morphological characteristics were observed by light (Nikon ECLIPSE E200) and scanning electron microscopy (SEM) (Hitachi SU8010) using cultures grown on ISP 3 medium at 28 °C for 6 weeks. Samples for scanning electron microscopy were prepared as described by Guan et al. (2015). Spore motility was assessed by light microscopic (Nikon ECLIPSE E200) observation of cells suspended in phosphate buffer (pH 7.0, 1 mM). Cultural characteristics were determined after 2 weeks at 28 °C using ISP media 2–7 (Shirling and Gottlieb 1966), nutrient agar (Waksman 1961), Bennett's agar (Jones 1949) and Czapek's agar (Waksman 1967). Colour determination was done with colour chips from the ISCC-NBS color charts (Kelly 1964). Growth at different temperatures (5, 10, 15, 20, 25, 28, 35, 37, 40 and 45 °C) was determined on ISP 3 medium after incubation for 14 days. The pH range for growth (pH 4–12, at intervals of 1 pH units) was tested in GY broth (Jia et al. 2013) using the buffer system described by Xu et al. (2005), and NaCl tolerance (0, 1, 3, 5, 7, 8, 9, 10, 11, 12 and 15% w/v) for growth were determined after 14 days growth in ISP 2 broth in shake flasks (250 rpm) at 28 °C. Hydrolysis of Tweens (20, 40 and 80) and production of catalase and urease were tested as described by Smibert and Krieg (1994). The utilisation of sole carbon and nitrogen sources, decomposition of cellulose, hydrolysis of starch and aesculin, reduction of nitrate, peptonisation of milk, liquefaction of gelatin and production of H_2S were examined as described previously (Gordon et al. 1974; Yokota et al. 1993).

Chemotaxonomic characterisation

Biomass for chemical studies was prepared by growing the organisms in GY broth in shake flasks at 28 °C for 7 days. Cells were harvested by centrifugation, washed with distilled water and freeze-dried. The isomer of diaminopimelic acid in the cell wall hydrolysates was derivatised as described in McKerrow et al. (2000) and analysed by a HPLC method (McKerrow et al. 2000) using an Agilent TC-C18 Column (250 × 4.6 mm i.d. 5 μm) with a mobile phase consisting of acetonitrile: 0.05 mol l^{-1} phosphate buffer pH 7.2 (15:85, v/v) at a flow rate of

$0.5\ ml\ min^{-1}$. The peak detection used an Agilent G1321A fluorescence detector with a 365 nm excitation and 455 nm long pass emission filters. The whole cell sugars were analysed according to the procedures developed by Lechevalier and Lechevalier (1980). The polar lipids were examined 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 analyzed by a HPLC–UV method (Wu et al. 1989). To determine cellular fatty acid composition, strain NEAU-GS4^T was cultivated in GY broth 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 gene sequence

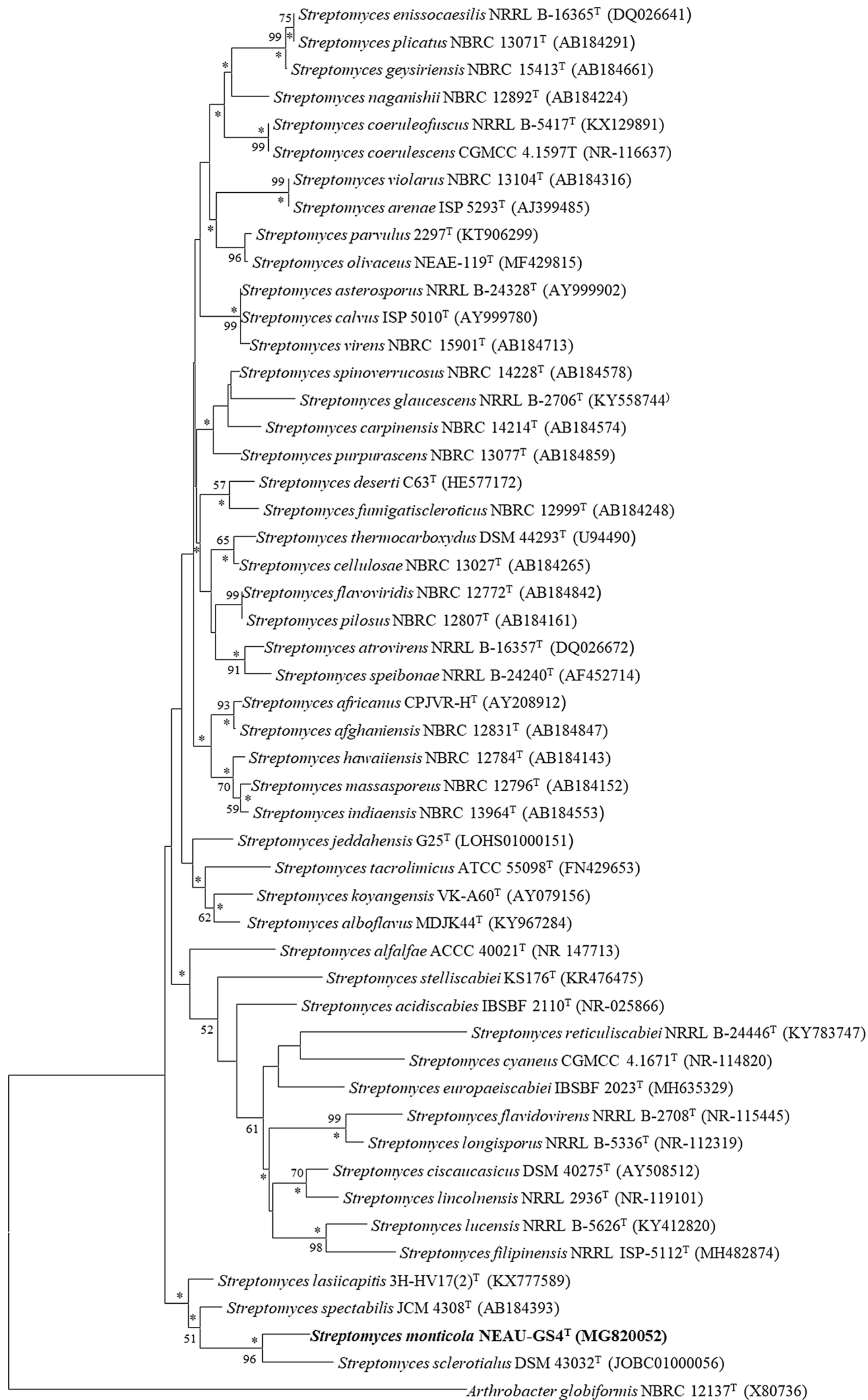
Extraction of genomic DNA, PCR amplification of the 16S rRNA gene sequence and sequencing of PCR products were carried out using a standard procedure (Kim et al. 2000). The PCR product was purified and cloned into the vector pMD19-T (Takara) and sequenced using an Applied Biosystems DNA sequencer (model 3730XL). The almost full-length 16S rRNA gene sequence of strain NEAU-GS4^T (1520 bp) was obtained and aligned with multiple sequences obtained from the GenBank/EMBL/DDBJ databases using Clustal X 1.83 software. Phylogenetic trees were constructed with neighbor-joining (Saitou and Nei 1987) and maximum-likelihood (Felsenstein 1981) algorithms using molecular evolutionary genetics analysis (MEGA) software version 6.06 (Tamura et al. 2013). The stability of the topology of the phylogenetic trees was assessed using the bootstrap method with 1000 replicates (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). Pairwise alignment analysis of 16S rRNA gene sequence similarities between strains were calculated on the Ezbiocloud server (Kim et al. 2012). The *gyr*B gene was amplified with primers PF-1 and PR-2 (Hatano et al. 2003) under the PCR program used for the 16S rRNA gene. The *atp*D, *rec*A, *rpo*B and *trp*B genes were performed using primers and amplification conditions described by Guo et al. (2008). MLSA was conducted as described previously (Rong et al. 2009). Briefly, sequences of each locus were aligned using MEGA 6.06 software, and trimmed manually at the same position before being used for further analysis. Trimmed sequences of the five housekeeping genes were concatenated head-to-tail in-frame in the order *atp*D (211 bp)-*gyr*B (383 bp)-*rec*A (194 bp)-*rpo*B (449 bp)-*trp*B (220 bp). Phylogenetic analysis was performed as described above. Strain NEAU-GS4^T, *S. spectabilis* JCM 4308^T, *S. sclerotialus* DSM 43032^T and *S. lasiicapitis* 3H-HV17(2)^T were included in the phylogenetic trees.

DNA base composition and DNA–DNA hybridization

The G + C content of strain NEAU-GS4^T was determined by using the thermal denaturation (Tm) method (Mandel and Marmur 1968) with *Escherichia coli* JM109 DNA used as the control. DNA–DNA relatedness tests were carried out between the strain and its close phylogenetic relatives according to De Ley et al. (1970), 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) under condition of the modifications described by Huss et al. (1983). The DNA samples used for hybridization were diluted to OD260 around 1.0 using 0.1 × SSC (saline sodium citrate buffer), 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 triplicate in 2 × SSC at 70 °C with three replications and the DNA–DNA relatedness value was expressed as a mean value.

Results and discussion

The almost complete 16S rRNA gene sequence of strain NEAU-GS4^T (1520 bp) was determined and deposited with the accession number MG820052 in the GenBank/EMBL/DDBJ databases. Comparative 16S rRNA gene sequence analysis by using the EzBioCloud server showed that the strain belongs to the genus *Streptomyces*. Strain NEAU-GS4^T showed close relationships with *S. spectabilis* JCM 4308^T



Streptomyces enissocaesilis NRRL B-16365^{T} (DQ026641)
Streptomyces plicatus NBRC 13071^{T} (AB184291)
Streptomyces geysiriensis NBRC 15413^{T} (AB184661)
Streptomyces naganishii NBRC 12892^{T} (AB184224)
Streptomyces coeruleofuscus NRRL B-5417^{T} (KX129891)
Streptomyces coerulescens CGMCC 4.1597T (NR-116637)
Streptomyces violarus NBRC 13104^{T} (AB184316)
Streptomyces arenae ISP 5293^{T} (AJ399485)
Streptomyces parvulus 2297^{T} (KT906299)
Streptomyces olivaceus NEAE-119^{T} (MF429815)
Streptomyces asterosporus NRRL B-24328^{T} (AY999902)
Streptomyces calvus ISP 5010^{T} (AY999780)
Streptomyces virens NBRC 15901^{T} (AB184713)
Streptomyces spinoverrucosus NBRC 14228^{T} (AB184578)
Streptomyces glaucescens NRRL B-2706^{T} (KY558744)
Streptomyces carpinensis NBRC 14214^{T} (AB184574)
Streptomyces purpurascens NBRC 13077^{T} (AB184859)
Streptomyces deserti C63^{T} (HE577172)
Streptomyces fumigatiscleroticus NBRC 12999^{T} (AB184248)
Streptomyces thermocarboxydus DSM 44293^{T} (U94490)
Streptomyces cellulosae NBRC 13027^{T} (AB184265)
Streptomyces flavoviridis NBRC 12772^{T} (AB184842)
Streptomyces pilosus NBRC 12807^{T} (AB184161)
Streptomyces atrovirens NRRL B-16357^{T} (DQ026672)
Streptomyces speibonae NRRL B-24240^{T} (AF452714)
Streptomyces africanus CPJVR-H^{T} (AY208912)
Streptomyces afghaniensis NBRC 12831^{T} (AB184847)
Streptomyces hawaiiensis NBRC 12784^{T} (AB184143)
Streptomyces massasporeus NBRC 12796^{T} (AB184152)
Streptomyces indiaensis NBRC 13964^{T} (AB184553)
Streptomyces jeddahensis G25^{T} (LOHS01000151)
Streptomyces tacrolimicus ATCC 55098^{T} (FN429653)
Streptomyces koyangensis VK-A60^{T} (AY079156)
Streptomyces alboflavus MDJK44^{T} (KY967284)
Streptomyces alfalfae ACCC 40021^{T} (NR 147713)
Streptomyces stelliscabiei KS176^{T} (KR476475)
Streptomyces acidiscabies IBSBF 2110^{T} (NR-025866)
Streptomyces reticuliscabiei NRRL B-24446^{T} (KY783747)
Streptomyces cyaneus CGMCC 4.1671^{T} (NR-114820)
Streptomyces europaeiscabiei IBSBF 2023^{T} (MH635329)
Streptomyces flavidovirens NRRL B-2708^{T} (NR-115445)
Streptomyces longisporus NRRL B-5336^{T} (NR-112319)
Streptomyces ciscaucasicus DSM 40275^{T} (AY508512)
Streptomyces lincolnensis NRRL 2936^{T} (NR-119101)
Streptomyces lucensis NRRL B-5626^{T} (KY412820)
Streptomyces filipinensis NRRL ISP-5112^{T} (MH482874)
Streptomyces lasiicapitis 3H-HV17(2)^{T} (KX777589)
Streptomyces spectabilis JCM 4308^{T} (AB184393)
Streptomyces monticola NEAU-GS4^{T} (MG820052)
Streptomyces sclerotialus DSM 43032^{T} (JOBC01000056)
Arthrobacter globiformis NBRC 12137^{T} (X80736)
0.01

◀**Fig. 1** Neighbour-joining tree based on 16S rRNA gene sequences (1429 bp) showing the relationship between strain NEAU-GS4^T and members of the genus *Streptomyces*. Asterisks indicate branches that were also recovered using the maximum-likelihood method. Bootstrap values (expressed as percentages of 1000 replications) of above 50% are shown at branch points. *Arthrobacter globiformis* NBRC 12137^T (X80736) was used as the outgroup. Bar, 0.01 substitutions per nucleotide position

(98.8%), *S. sclerotialus* DSM 43032^T (98.3%) and *S. lasiicapitis* 3H-HV17(2)^T (98.0%). The phylogenetic tree demonstrated that the strain formed a cluster with the above-mentioned three strains based on the neighbor-joining algorithm (Fig. 1) and this relationship was also recovered by the maximum-likelihood algorithm (Supplementary Fig. S1). To further clarify the affiliation of strain NEAU-GS4^T, partial sequences of housekeeping genes including *atp*D, *gyr*B, *rec*A, *rpo*B and *trp*B were obtained. GenBank accession numbers of the sequences, the almost complete length of each gene and similarities of related species with strain NEAU-GS4^T are displayed in Table 1 (all sequences of *S. sclerotialus* DSM 43032^T were retrieved from the Genomes OnLine Database). In the present MLSA analysis, the neighbour-joining phylogenetic tree constructed from the concatenated sequence alignment (1476 bp) of five housekeeping genes (Fig. 2) suggested that this isolate forms a cluster with *S. spectabilis* JCM 4308^T, *S. lasiicapitis* 3H-HV17(2)^T, *Streptomyces alboflavus* MDJK44^T (with which it has 16S rRNA gene sequence similarity, 92.7%) and *Streptomyces alfalfae* ACCC 40021^T (94.8%); but *S. sclerotialus* DSM 43032^T is loosely associated with this lineage. Therefore, the MLSA analysis further indicated that the strain should be assigned to the genus *Streptomyces*. Moreover, the isolate NEAU-GS4^T was shown to have MLSA distances greater than 0.007 with all of these strains indicating that it forms the nucleus of a novel *Streptomyces* species (Labeda et al. 2017). To determine whether strain NEAU-GS4^T does indeed represent a novel genomic species, DNA–DNA hybridization was employed to further clarify the relatedness between strain NEAU-GS4^T and *S. spectabilis* JCM 4308^T, *S. sclerotialus* DSM 43032^T and *S. lasiicapitis* 3H-HV17(2)^T; the levels of DNA–DNA relatedness between them were 39.0 ± 5.8%, 39.1 ± 4.7% and 45.2 ± 3.1%., respectively. These values are below the threshold value of 70% recommended by Wayne et al. (1987) for assigning bacterial strains to the same genomic species.

Morphological observation of 6-week-old culture of strain NEAU-GS4^T growth on ISP 3 medium revealed that the strain forms well-developed, branched substrate hyphae and aerial mycelium that differentiate into straight or flexuous spore chains consisting of cylindrical spores. The spore surface was observed to be wrinkled (Fig. S2). Strain NEAU-GS4^T shows good growth on ISP 4 and ISP 3 media; moderate growth on Czapek's agar, ISP 1, ISP 2 and ISP 7 media; poor growth on ISP 5, ISP 6 and nutrient agar; and no growth on Bennett's medium (Fig. S3). The detailed cultural characteristics of the strain NEAU-GS4^T are shown in Table 2. No diffusible pigment was observed on any of the tested media. The strain can utilise L-arabinose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannitol, D-mannose, *myo*-inositol, raffinose, L-rhamnose, D-sorbitol and sucrose but not dulcitol, D-ribose or D-xylose as sole carbon sources. L-Alanine, L-asparagine, L-aspartic acid, glycine, L-glutamic acid, L-glutamine, L-proline,

Table 1 GenBank accession numbers of the gene sequences determined, the almost complete length of each gene and similarities between strain NEAU-GS4^T and the type strains of closely related species of the genus *Streptomyces*

Locus	GenBank accession numbers			Length (bp)			Similarity (%) with strain NEAU-GS4^T		
	1	2	3	1	2	3	2	3	4
16S rRNA	MG820052	EU521692	KX777589	1520	1457	1521	98.8	98.0	98.3
*atp*D	MH651784	MH651783	MH651782	865	885	875	83.4	79.5	74.3
*gyr*B	MH024392	KY275268	KY229066	1185	1185	1195	87.1	87.8	86.6
*rec*A	MH651787	MH651786	MH651785	854	877	868	92.3	92.4	90.5
*rpo*B	MH651790	MH651789	MH651788	919	927	888	90.2	88.4	88.9
*trp*B	MH651793	MH651792	MH651791	325	829	793	89.6	88.5	88.2

Strains: 1, NEAU-GS4^T; 2, *S. spectabilis* JCM 4308^T; 3. *S. lasiicapitis* 3H-HV17(2)^T; 4, *S. sclerotialus* DSM 43032^T

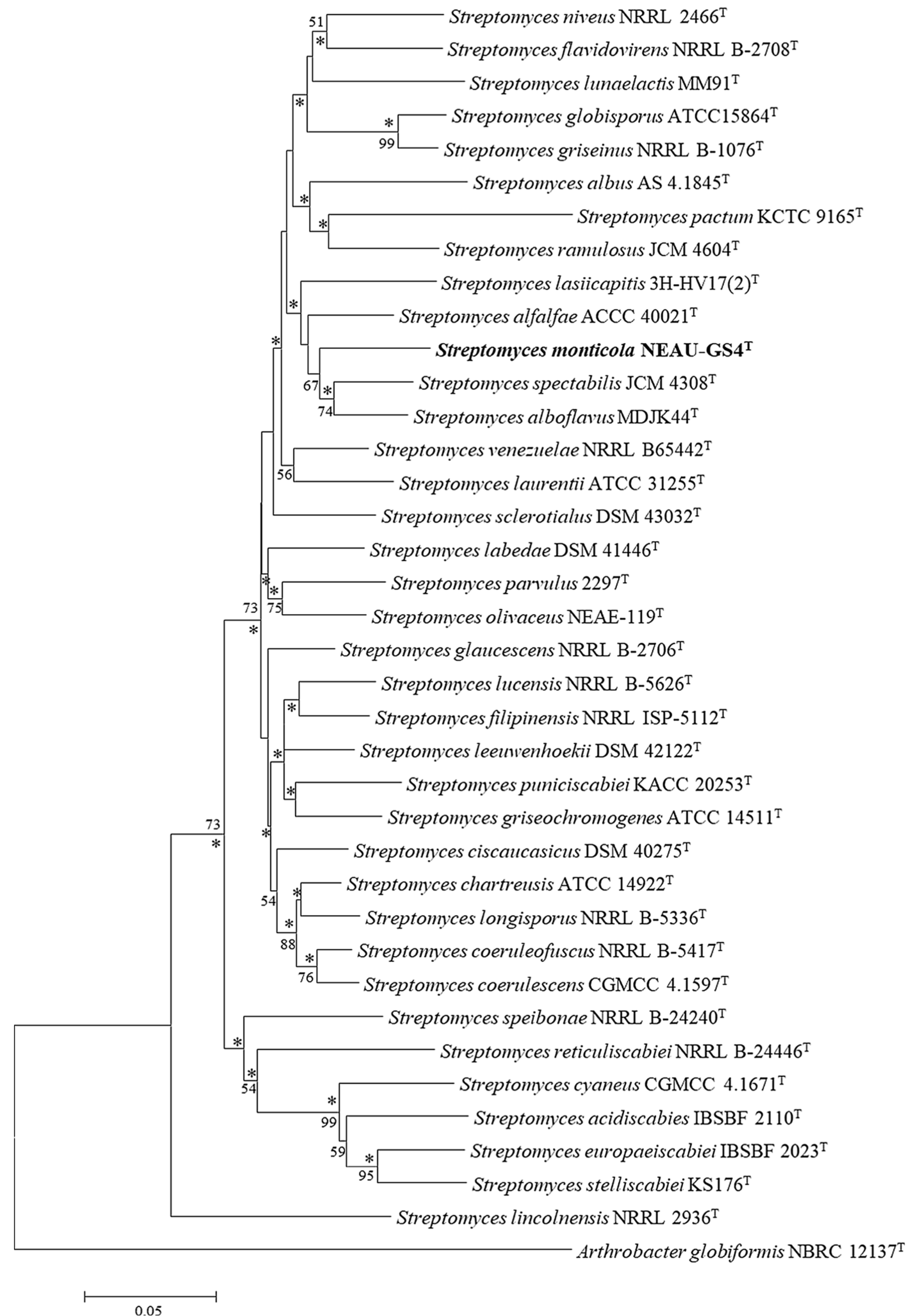


Fig. 2 Neighbour-joining tree based on MLSA analysis of the concatenated partial sequences (1476 bp) from five housekeeping genes (*atp*D, *gyr*B, *rec*A, *rpo*B and *trp*B) of isolate NEAU-GS4^T (in bold) and related taxa. Only bootstrap values above 50% (percentages of 1000 replications) are indicated. Asterisks indicate branches also recovered in the maximum-likelihood tree; Bar, 0.05 nucleotide substitutions per site

L-serine, L-threonine and L-tyrosine are utilised as sole nitrogen sources, but not L-arginine or creatine. A comparison of phenotypic characteristics between the isolate and its close relatives was performed to differentiate them (Table 3). Strain NEAU-GS4^T can be distinguished from *S. spectabilis* JCM 4308^T, *S. sclerotialus* DSM 43032^T and *S. lasiicapitis* 3H-HV17(2)^T by the range of growth temperature, pH and tolerance range of NaCl. Moreover, differences including utilisation of L-arginine and creatine also help to differentiate the isolate from its three related species.

Strain NEAU-GS4^T was found to exhibit a range of chemotaxonomic properties that are consistent with

Table 2 Growth and cultural characteristics of strain NEAU-GS4^T after streak plating for 2 weeks at 28 °C

Agar Media	Growth	Aerial mycelium	Aerial mycelium colour	Substrate mycelium colour
ISP 1	Moderate	−	None	Brilliant yellow
ISP 2	Moderate	−	None	Brilliant greenish yellow
ISP 3	Good	+	White	Moderate pink
ISP 4	Good	+	Grayish olive	Dark olive
ISP 5	Poor	−	None	White
ISP 6	Poor	−	None	Strong greenish yellow
ISP 7	Moderate	+	Strong greenish yellow	Light greenish yellow
Czapek's	Moderate	+	White	Bluish white
Nutrient	Poor	−	None	Strong yellow
Bennett's	−	−	−	−

+, presence; −, absence

the description of the genus *Streptomyces*. It contains *LL*-diaminopimelic acid as cell wall diamino acid, indicating that the strain is of cell wall chemotype I (Lechevalier and Lechevalier 1970a, b). The whole cell sugars were found to contain glucose and ribose. The polar lipids were found to consist of diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylinositolmannosides and an unidentified phospholipid (phospholipid type II sensu Lechevalier et al. 1977) (Fig. S4). The cellular fatty acids were identified as anteiso-$C_{15:0}$ (20.1%), iso-$C_{16:0}$ (18.2%), anteiso-$C_{17:0}$ (13.3%), $C_{16:0}$ (7.9%), $C_{15:0}$ (7.6%), $C_{18:1}$ ω7C (6.1%), iso-$C_{14:0}$ (5.6%), $C_{17:1}$ ω8C (4.8%), $C_{18:0}$ (4.7%), $C_{17:0}$ (4.1%), $C_{17:1}$ ω7C (3.6%), $C_{16:1}$ ω7C (2.4%) and $C_{14:0}$ (1.0%). The menaquinones were found to be MK-9(H_6) (40.7%), MK-9(H_8) (25.6%), MK-9(H_4) (23.0%) and MK-9(H_2) (10.7%). The DNA G + C content of strain NEAU-GS4^T was determined to be 71.1 mol %.

In conclusion, the differences in various characteristics showed that strain NEAU-GS4^T is phenotypically distinct from its phylogenetic relatives *S. spectabilis* JCM 4308^T, *S. sclerotialus* DSM 43032^T and *S. lasiicapitis* 3H-HV17(2)^T. Therefore, it is evident that strain NEAU-GS4^T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces monticola* sp. nov. is proposed. The Digital Protologue (Rosselló-Móra et al. 2017) TaxoNumber for strain NEAU-GS4^T is TA00455.

Description of *Streptomyces monticola* sp. nov.

Streptomyces monticola (mon.ti'co.la. L. n. *mons, montis* mountain; -*cola* from L. n. *incola* an inhabitant; N.L. masc. n. *monticola* mountain-dwelling referring to the isolation source of soil from Mount Song).

Gram-stain positive aerobic actinomycete that forms well-developed, branched substrate hyphae and aerial mycelium that differentiates into straight or flexuous spore chains consisting of cylindrical spores; the spore surface is wrinkled. Growth occurs at 10–37 °C and pH values of 6–10. Optimal temperature and pH for growth are 28 °C and 7.0, respectively. Tolerates up to 11.0% NaCl, with optimum concentration between 0 and 8.0% (w/v). Positive for hydrolysis of aesculin, starch, urease and Tweens (20 and 40), liquefaction of gelatin and coagulation and peptonisation of milk. Negative for hydrolysis of cellulose, production of H_2S, reduction of nitrate and degradation of Tween 80. Whole cell sugars include glucose and ribose. The polar lipids consist of diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylinositolmannosides and an unidentified phospholipid. The major fatty acids (> 10%) are anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$. The G + C content of the DNA of the type strain is 71.1 mol%.

The type strain is NEAU-GS4^T (=CGMCC 4.7467^T = DSM 105116^T), isolated from soil collected from Mount Song. The GenBank/EMBL/DDBJ databases accession number of the 16S rRNA NEAU-GS4^T is MG820052.

Table 3 Differential characteristics of strain NEAU-GS4^T compared with the closely related species *S. spectabilis* JCM 4308^T, *S. sclerotialus* DSM 43032^T and *S. lasiicapitis* 3H-HV17(2)^T

Characteristic	1	2	3	4
Utilisation as sole carbon source				
D-ribose	−	+	−	+
Maltose	+	+	−	+
D-mannose	+	−	+	+
D-sorbitol	+	−	−	+
Lactose	+	+	−	+
myo-inositol	+	−	+	+
Utilisation as sole nitrogen source				
L-arginine	−	+	+	+
Glycine	+	+	−	+
L-glutamine	+	+	−	+
L-asparagine	+	+	−	+
L-tyrosine	+	+	−	+
L-serine	+	−	−	+
L-threonine	+	+	−	+
L-proline	+	−	−	+
Creatine	−	+	+	+
Liquefaction of gelatin	+	+	−	−
Hydrolysis of				
Aesculin	+	−	+	+
Tween 20	+	−	+	+
Tween 40	+	+	−	−
Tween 80	−	−	+	+
Growth temperature (°C)	10–37	15–40	20–40	10–37
Growth pH	6–10	5–9	6–10	4–9
Maximum NaCl tolerance (%, w/v)	11	12	5	9
Menaquinones	MK-9(H_8) MK-9(H_6) MK-9(H_4) MK-9(H_2)	MK-9(H_8) MK-9(H_6) MK-9(H_4)	MK-9(H_8) MK-9(H_6) MK-9(H_4)	MK-9(H_8) MK-9(H_6) MK-9(H_4)
Polar lipids	DPG, PME, PE, PIM, PL	DPG, PME, PE, PIM, UL	DPG, PE, PIM, PL, UL	DPG, PE, PI
Whole-cell sugar	Glucose, ribose	Glucose, ribose	Glucose, ribose	Glucose, ribose
Fatty acid (> 10%)	anteiso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$	anteiso-$C_{15:0}$, iso-$C_{16:0}$, $C_{15:0}$, anteiso-$C_{17:0}$, $C_{16:0}$	$C_{16:0}$, $C_{17:1}$ ω7C, $C_{15:0}$, anteiso-$C_{15:0}$	iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$, $C_{16:0}$

Reference strains: 1, NEAU-GS4^T; 2, *S. spectabilis* JCM 4308^T; 3, *S. sclerotialus* DSM 43032^T; 4, *S. lasiicapitis* 3H-HV17(2)^T. All data are from this study

+, positive; −, negative

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Author's contributions DL performed the laboratory experiments, analyzed the data, and drafted the manuscript. LH contributed to the biochemical characterisation. JZ contributed to the polyphasic taxonomy. HJ contributed to the morphological analyzes. SJ contributed to the fatty acids determination. XG participated in the discussions of each section of experiments. XW and WX designed the experiments and revised the manuscript.

Compliance with ethical standards

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

Ethical standards 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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