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ORIGINAL PAPER

Streptomyces akebiae sp. nov., a novel actinomycete isolated from rhizosphere soil of *Akebia trifoliate*

Ping Mo · Kaiqin Li · Jinhua Zhou · Fumin Zhou · Yazhi Chen · Xiaojia Lin · Xiaoyu Liu · Kerui Huang · Wansheng Zou · Jian Gao

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Abstract A novel actinomycete strain, designated as MG28^T, was isolated from rhizosphere soil of *Akebia trifoliat*e. The taxonomic position of the strain was investigated by using a polyphasic approach. BLAST search of the full-length 16S rRNA gene sequence of strain MG28^T indicated it represented a member of the genus *Streptomyces*, and displayed 99.03%, 98.90%, 98.90%, 98.89%, 98.83% and less than 98.70% sequence similarities with *S. phaeolivaceus* GY16^T, *S. deccanensis* KCTC 19241^T, *S. europaeiscabiei* KACC 20186^T, *S. fructofermentans* CGMCC 4.1593^T, *S. scabiei* NRRL B-16523^T and other species of the genus *Streptomyces* with validly published names, respectively. Phylogenomic analysis indicated that strain MG28^T was closely related to *Streptomyces deccanensis* KCTC 19241^T. However, the average nucleotide identity values and the digital DNA-DNA hybridization values between them indicated that strain MG28^T represented a distinct species. Furthermore, strain MG28^T was also distinctly differentiated from strain KCTC 19241^T by morphological, physiological and biochemical characteristics. Therefore, strain MG28^T (=MCCC 1K06895^T=JCM 34922^T) represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces akebiae* sp. nov. is proposed.

Keywords *Streptomyces akebiae* sp. nov. · *Akebia trifoliate* · *Streptomycetaceae* · Novel species · Polyphasic taxonomy

Ping Mo and Kaiqin Li have contributed equally.

The GenBank/EMBL/DDBJ accession numbers for the full-length 16S rRNA gene sequence and genome sequence of strain MG28^T and *Streptomyces deccanensis* KCTC 19241^T are OM368590 and OM807210, and CP080647 and CP092431, respectively.

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P. Mo · J. Zhou · F. Zhou · Y. Chen · X. Lin · X. Liu · K. Huang · W. Zou (✉)
Hunan Provincial Key Laboratory for Health Aquaculture and Product Processing in Dongting Lake Area, Hunan Provincial Key Laboratory for Molecular Immunity Technology of Aquatic Animal Diseases, Zoology Key Laboratory of Hunan Higher Education, College of Life and Environmental Sciences, Changde innovation team of Wetland Biology and Environmental Ecology, Hunan University of Arts and Science, Changde 415000, Hunan, China
e-mail: zwsksy@163.com

K. Li
Hunan Institute of Agricultural Biotechnology, Changsha 410000, China

J. Gao (✉)
School of Life and Health Sciences, Hunan University of Science and Technology, Xiangtan 411201, China
e-mail: xtgojian@hnust.edu.cn

Abbreviations

MCCC Marine Culture Collection of China
ISP International Streptomyces Project

Introduction

Nowadays, plants and animals, as medicine source, play an important role (Alves 2012; Ho 2005), but it can't be ignored that microorganisms are also significant source of pharmaceutically drugs (Bérdy 2012; Debbab et al. 2010; Waditee-Sirisattha et al. 2016). In 2010, it was reported that more than 33,000 microbial secondary metabolites had been discovered. Among these bioactive compounds, approximately 13,600 were produced by actinomycetes, in which about 75% was produced by *Streptomyces* (Olano et al. 2009; Solecka et al. 2012). So, in recent decades, *Streptomyces* has gained more and more attention for their potential ability to produce new natural products (Bouizgarne et al. 2009; Goodfellow and Fiedler 2010). These reports encouraged us to search for novel *Streptomyces* with potentially useful applications in all kinds of samples. In previous studies, many novel *Streptomyces* such as *S. rhizosphaericola* (Vargas Hoyos et al. 2019), *S. tritici* (Zhao et al. 2018), *S. triticagri* (Han et al. 2020), *S. triticirhizae* (Han et al. 2020) and *S. inhibens* (Jin et al. 2019), *S. fagopyri* (Guo et al. 2020) and *S. broussonetiae* (Mo et al. 2020) have been isolated from the rhizosphere soil of plants. In recent years, during the process of investigating the diversity and function of microbes from the rhizosphere soil of *Akebia trifoliat*e, one of strains, designated as MG28^T, showed antifungal activity against plant fungi. The aim of the present work was to determine the taxonomic status of this strain. The results of polyphasic taxonomic studies indicated that strain MG28^T represents a novel species of genus *Streptomyces*, for which the name *Streptomyces akebia* sp. nov. is proposed.

Materials and methods

Isolation and maintenance of the organism

The rhizosphere soil sample of *Akebia trifoliat*e was collected from Changde city located in the northwest of Hunan Province, China (N29° 28′ 30.87″, E111° 27′ 57.70″). Strain MG28^T was isolated using the methods as described by Mo et al. (2018). Strain MG28^T was stored for short term deposition on ISP 4 agar medium (Shirling and Gottlieb 1966) slopes at 4 °C and suspended in sterile 30% (w/v) glycerol solution for long term deposition at − 80 °C. The type strain, *Streptomyces deccanensis* KCTC 19241^T was purchased from the KCTC (Korean Collection for Type Cultures, Daejeon, Republic of Korea). Reference strain was grown under the same conditions for comparative testing.

Morphological, cultural and physiological characteristics

Cell morphology of strain MG28^T were observed by scanning electron microscope (FEI-Quanta 450, America) after incubation on Gause's synthetic agar medium (Atlas 1993) for 21 days at 28 °C. The color of colonies and soluble pigments were observed on Gause's synthetic medium and ISP media (Shirling and Gottlieb 1966) after incubation at 28 °C for 21 days. Colors of substrate mycelia, aerial hyphae and soluble pigments were determined by the Color Standards and Color Nomenclature (Ridgway 1912). Growth at different temperatures (4, 10, 15, 20, 25, 30, 35, 40 and 45 °C) and the concentrations of NaCl (0–14%, w/v with an interval of 1% unit) was tested on ISP2 medium at 28 °C for 14 days. The pH range (pH 4.0–13.0 at intervals of 1.0 pH unit) for growth was tested by using ISP 2 as the basal medium using the buffer system as described by Xu et al. (2007). Carbon source utilization was tested by using ISP 9 medium (Shirling and Gottlieb 1966) supplemented with 0.5% carbon sources. The utilization of nitrogen sources was performed as described previously (Shirling and Gottlieb 1966). The other physiological characteristic including gelatin liquefaction, nitrate reduction, starch and aesculin hydrolysis, degradation tests for Tweens (20, 40, 60 and 80) were carried out according to the methods described by Xu et al. (2007) and Ruan and Huang (2011). The experiments were carried out in triplicate.

Chemotaxonomic characterisation

Biomass for chemotaxonomic analysis was collected by centrifugation from cultures grown in tryptic soy broth medium (TSB medium) in shake flasks for

7 days at 28 °C and then washed twice with distilled water. Cellular fatty acid composition analysis was carried out by China Center of Industrial Culture Collection (CICC) according to the protocol of the Sherlock Microbial Identification system (MIDI system; http://www.midi-inc.com/) and analyzed by GC (6968; Hewlett Packard) using the Microbial Identification software package (MIDI 2005). The isomers of diaminopimelic acid analysis and sugar analysis in whole-cell hydrolysates were performed according to the procedures described by Hasegawa et al. (1983) and Lechevalier and Lechevalier (1970). Menaquinones were extracted according to the method of Collins et al. (1977) and analysed by HPLC (Kroppenstedt 1985). The polar lipids were extracted, separated and analysed as described by Komagata and Suzuki (1987).

Phylogenetic analysis and genomic DNA-DNA correlation analysis

Genomic DNA extraction and PCR amplification of the 16S rRNA gene were performed as described by Weisburg et al. (1991) and Lane (1991). The PCR product was purified using a Mag Extractor PCR and Gel Clean up kit (TOYOBO) according to the manufacturers' instructions and sequenced directly using an automated DNA sequencing system (ABI 3730XL; Applied Biosystems) by Sangon Biotech (Shanghai). Whole-genome sequencing of strain MG28^T and *S. deccanensis* KCTC 19241^T was performed by Wuhan Benagen Technology Co., Ltd (Hubei, China) using a Nanopore PromethION sequencing system. The sequenced reads were assembled using SOAPdenovo (version 2.04) software (Xie et al. 2014). The 16S rRNA gene sequence of strain MG28^T was compared with public databases and EzBioCloud database (http:// www.ezbiocloud.net/eztaxon; (Yoon et al. 2017). The genome sequences of strain MG28^T and the closely related type strains with validly published names were used for reconstructing phylogenetic trees. Phylogenomic analysis was carried out using the Type (Strain) Genome Server (https://tygs.dsmz.de/) (Meier-Kolthoff and Göker 2019). The average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values between the genomes of strain MG28^T and its relatives were calculated using the JSpeciesWS online service (Richter et al. 2016) and the genome-to-genome distance calculator (Meier-Kolthoff et al. 2013), respectively. For calculating dDDH value, Formula 2 was used. The G+C content of the genomic DNA of strain MG28^T was deduced from the genomic data.

Results and discussion

Morphological and physiological characterization of the strain MG28^T

The morphological characteristics showed that strain MG28^T had the typical characteristics of the genus *Streptomyces*. Cells of strain MG28^T was Gram-stain-positive and aerobic. Strain MG28^T formed an extensively branched substrate hyphae and aerial mycelium on Gause's synthetic agar medium, and produced straight to flexuous spore chains consisting of rod spores with smooth-surfaced (Fig. 1). Strain MG28^T was observed to grow well on all tested media. Melanin and diffusible pigment were produced on ISP 6 and ISP 7 agar media, respectively. Other cultural characteristics of strain MG28^T were shown in Table 1. Strain MG28^T was found to grow at 4–45 °C (optimum, 28 °C), pH 6.0–11.0 (optimum, pH 7.0) and tolerate up to 5.0% (w/v) NaCl. Detailed physiological characteristics about sole carbon source

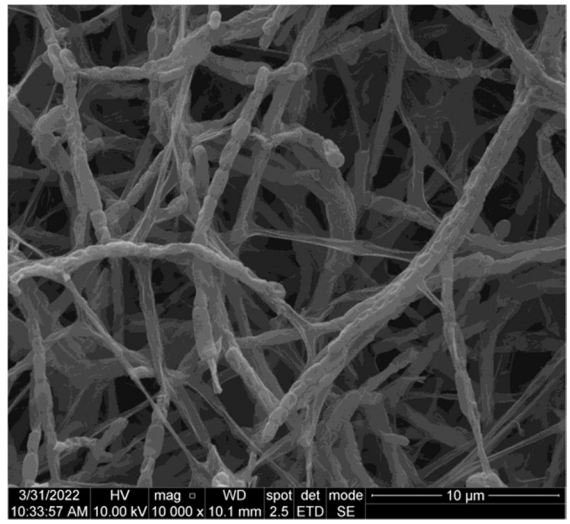

Fig. 1 Scanning electron micrograph of strain MG28^T grown on Gause's synthetic agar medium at 28 °C after incubation 21 days

Table 1 Cultural characteristics between strains MG28^T and *S. deccanensis* KCTC 19241^T in different medium after incubation for 21 days at 28 °C

Characteristics	Strain MG28^T	KCTC 19241^T
Color of AM on No. 1	Gray	White
Color of SM on No. 1	Yellow	Kaiser brown
Diffusible pigment on No. 1	None	Apricot buff
Color of AM on ISP 2	Deep grayish olive	White to gray
Color of SM on ISP 2	Hair brown	Aniline yellow
Diffusible pigment on ISP 2	None	Yellow
Color of AM on ISP 3	Gray	Gray
Color of SM on ISP 3	Yellow	Apricot yellow
Diffusible pigment on ISP 3	None	None
Color of AM on ISP 4	Dark grayish olive	White
Color of SM on ISP 4	Grayish olive	Deep olive buff
Diffusible pigment on ISP 4	None	None
Color of AM on ISP 5	Yellow to white	White and buckthorn brown
Color of SM on ISP 5	Yellow	Buckthorn brown
Diffusible pigment on ISP 6	Black	Black
Color of AM on ISP 7	Gray	White
Color of SM on ISP 7	Brown	Brown
Diffusible pigment on ISP 7	Black	Black

No. 1, Gause's synthetic medium

AM aerial mycelium; SM substrate mycelium

utilization about strains are presented in the species description.

Chemotaxonomic characteristics

The chemotaxonomic studies revealed that strain MG28^T exhibited characteristics typical of members of the genus *Streptomyces*. For example, the cell wall contained LL-diaminopimelic acid as the diagnostic amino acid, and the whole-cell hydrolysates were galactose and glucose. The predominant cellular fatty acids (>5%) of strain MG28^T were Sum in Feature 3 ($C_{16:1\omega7C}$/$C_{16:1\omega6C}$) (19.7%), *iso*-$C_{16:0}$ (18.9%), *anteiso*-$C_{15:0}$ (17.8%), $C_{16:0}$ (7.0%), *iso*-$C_{15:0}$ (5.3%) and *anteiso*-$C_{17:0}$ (5.2%). Other fatty acids present in smaller amounts (>1%) were *iso*-$C_{14:0}$ (4.8%), *anteiso*-$C_{17:1\omega5C}$ (2.9%), $C_{14:0}$ (2.9%), *iso* H-$C_{16:1}$ (2.3%), Sum in Feature 9 (10-methyl $C_{16:0}$) (2.0%), *cyclo*-$C_{17:0}$ (1.4%) and *anteiso*-$C_{13:0}$ (1.0%). The menaquinones were MK-9 (4.6%), MK-9(H_2) (30.1%), MK-9(H_4) (32.8%), MK-9(H_6) (18.3%) and MK-9(H_8) (12.9%), which is also typical of members of the genus *Streptomyces*. The DNA G+C content of strain MG28^T was 70.8%, within the range 69–78% of the member of the genus *Streptomyces* (Wright and Bibb 1992). The polar lipids consisted of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylinositol mannosides (PIM), phosphatidyl glycerol (PG) and unidentified spots (L1) (Fig. S1, available in the online version of this paper).

Phylogenic analysis

Based on the full-length 16S rRNA gene sequence (1527 bp) analysis, strain MG28^T represented a member of the genus *Streptomyces*, and shared 99.03% similarity with *S. phaeolivaceus* GY16^T, 98.90% similarities with *S. deccanensis* KCTC 19241^T and *S. europaeiscabiei* KACC 20186^T, 98.89% similarity with *S. fructofermentans* CGMCC 4.1593^T, 98.83% similarity with *S. scabiei* NRRL B-16523^T and less than 98.7% similarity with other type species of the genus *Streptomyces*. Considering that the phylogenomic analysis exhibited better resolution than the phylogenetic analysis based on 16S rRNA gene sequence (Rodriguez et al. 2018), so, in the present work, the phylogenomic analysis was carried out in order to clarify taxonomic status of strain MG28^T. It was shown in Fig. 2, strain MG28^T was clustered together with *S. deccanensis* KCTC 19241^T, suggesting that it was closely related to *S. deccanensis* KCTC 19241^T. However, the ANIm/ANIb and dDDH values between strain MG28^T and *S. deccanensis* KCTC

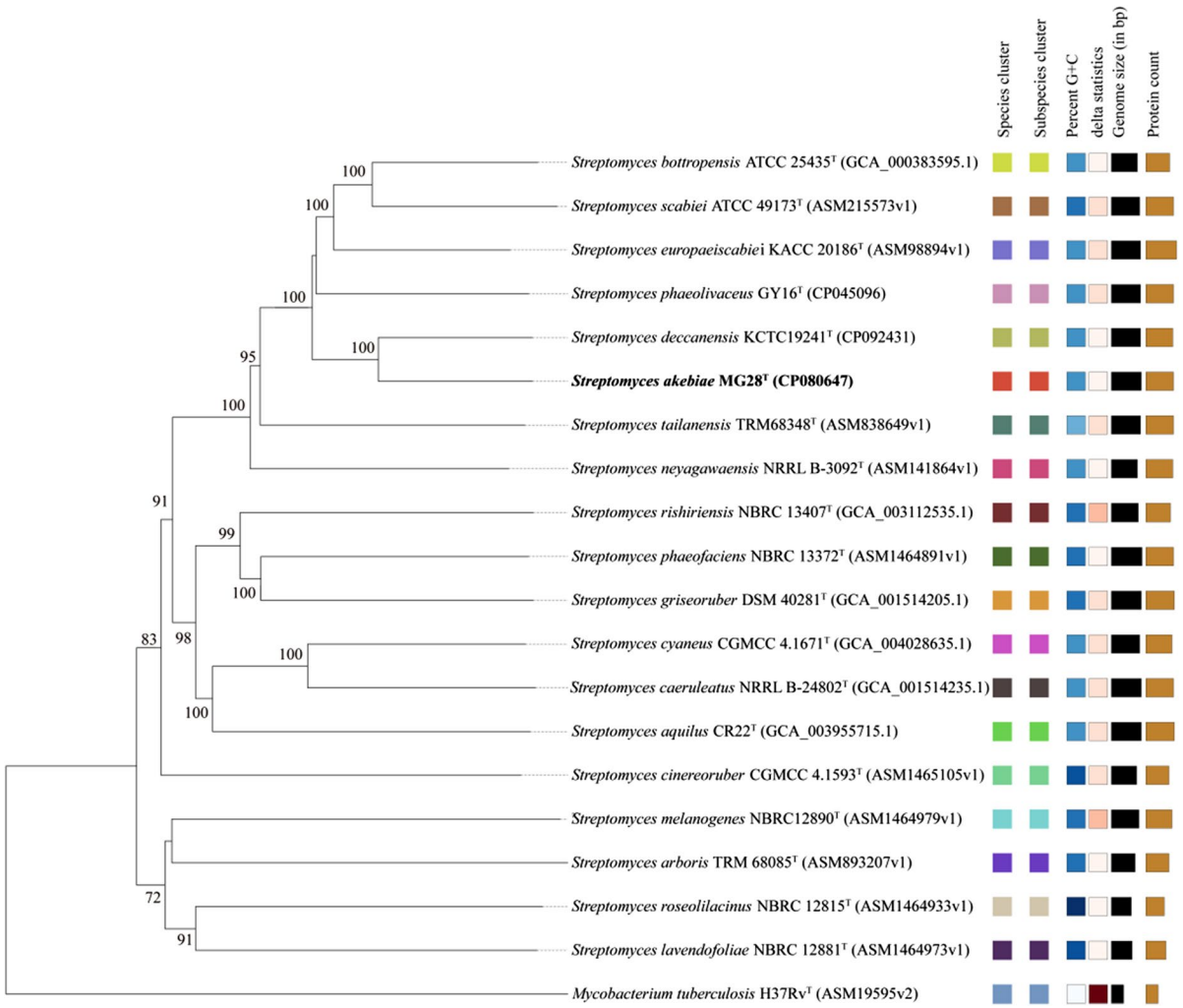

Fig. 2 Phylogenetic tree based on whole genome sequences of MG28^T and related reference strains. Tree inferred with FastME 2.1.6.1 (Vincent et al. 2015) from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d5. The numbers above branches are GBDP pseudo-bootstrap support values > 60% from 100 replications, with an average branch support of 96.0%. The tree was rooted at the midpoint (Farris 1972)

19241^T were 92.9/91.4% and 47.0%, much less than 95–96% and 70% cut-off points recommended for delineating species (Richter and Rosselló-Móra 2009; Chun et al. 2018). In addition, the cultural, physiological and biochemical characteristics were dissimilar enough to distinguish strain MG28^T from *S. deccanensis* KCTC 19241^T (Tables 1 and 2). For example, strain MG28^T forms a gray aerial mycelium and yellow substrate mycelium with no diffusible pigment on Gause's synthetic medium, while *S. deccanensis* KCTC 19241^T develops white aerial mycelium and kaiser brown substrate mycelium with apricot buff diffusible pigment. In addition, the appearance of the straight to flexuous spore's chain with smooth surfaces of strain MG28^T is clearly distinguishable from the straight spore's chain with hairy surfaces of *S. deccanensis* KCTC 19241^T. Meanwhile, according to the description of Stackebrandt and Ebers (2006), if 16S sequence similarity between two strains ≥ 98.7%, ANI or dDDH values need to be calculated to evaluate their DNA-DNA relatedness in delineating new species. In the present work, ANIm/ANIb and dDDH

Table 2 Characteristics differentiating strains MG28^T and *S. deccanensis* KCTC 19241^T

Characteristics	1	2
Spore surface	Smooth	Hairy
Spore chain	Straight to flexuous	Straight
Aesculin hydrolysis	+	−
Tween 20 decomposition	−	+
Tween 60 decomposition	−	+
Tween 80 decomposition	−	+
Urease	+	−
Growth at/with:		
Temperature	4–45	28–45
Tolerance to NaCl	0–5.0	0–7.0
pH	6.0–11.0	7.0–12.0
Use of carbon sources:		
Cellobiose	+	−
Glycerol	+	−
L-arabinose	−	+
Maltose	−	−
Myo-inositol	−	+
Use of nitrogen sources:		
Creatine	−	+
L-arginine	+	−
L-cystine	+	−
L-methionine	−	+
L-serine	−	−
L-valine	−	+
Major fatty acids (> 5.0% of total)		
iso-$C_{15:0}$	5.3	7.1
anteiso-$C_{15:0}$	17.8	14.6
$C_{16:0}$	7.0	8.3
iso-$C_{16:0}$	18.9	17.3
$C_{16:1}$-*Cis* 9	−	14.6
anteiso-$C_{17:0}$	5.2	–
Sum in Feature 3	19.7	−

1, strain MG28^T; 2, *S. deccanensis* KCTC 19241^T; −, negative or not detect; +, positive. All data are from this study. All strains positive for nitrate reduction, gelatin liquefaction, urease and degradation of starch, negative for Tween 40 decomposition. All strains were able to use D-mannitol, D-ribose, D-xylose, L-phenylalanine, L-rhamnose and sucrose as a carbon source; All strains were able to use L-phenylalanine as a nitrogen source; Summed in Feature 3, $C_{16:1\omega7C}$/$C_{16:1\omega6C}$

values between strain MG28^T and *S. phaeolivaceus* GY16^T, between strain MG28^T and *S. europaeiscabiei* KACC 20186^T, between strain MG28^T and *S. fructofermentans* CGMCC 4.1593^T, between strain MG28^T and *S. scabiei* NRRL B-16523^T were 85.8–90.7%/(80.2–91.4%) and 23.2–38.8% (Table 3) [37, 38], respectively, well below the 95–96% and 70% cut-off point recommended for delineating prokaryote species. In conclusion, strain MG28^T represents a novel *Streptomyces* species, for which the name *Streptomyces akebiae* sp. nov. is proposed.

Description of *Streptomyces akebiae* sp. nov.

Streptomyces akebiae (a.ke'bi.ae. N.L. gen. n. akebiae referring to come from Akebia trifoliata)

Good growth on Gause's synthetic medium and ISP 2-7. The aerial mycelia are gray and the substrate mycelia are yellow on Gause's synthetic agar medium. The aerial mycelia differentiate into straight to flexuous spore chains (which are) consisting of rod spores with smooth-surfaced. Growth occurs at pH 6.0–11.0 (optimum, 7.0), 4–45 °C (optimum, 28 °C) and 0–5.0% NaCl (w/v). Positive in tests for aesculin hydrolysis, nitrate reduction, gelatin liquefaction, urease and degradation of starch, negative for Tweens (20, 40, 60 and 80) decomposition. Cellobiose, D-mannitol, D-ribose, D-xylose, glycerol, L-rhamnose, and sucrose can be used as sole carbon source for growth, but not L-arabinose, maltose or *myo*-inositol. L-arginine, L-cystine and L-phenylalanine can be utilized as sole nitrogen source, but not for creatine, L-methionine, L-serine or L-valine. The cell wall contains alanine, glutamic acid, glycine and LL-diaminopimelic acid, and the whole-cell hydrolysates are galactose and glucose. The polar lipids are diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylinositol mannosides (PIM), phosphatidyl glycerol (PG) and unidentified spots (L1). The menaquinones are MK-9, MK-9(H_2), MK-9(H_4), MK-9(H_6) and MK-9(H_8). The major fatty acids (> 5%) are $C_{16:1\omega7C}$/$C_{16:1\omega6C}$, *iso*-$C_{16:0}$, *anteiso*-$C_{15:0}$, $C_{16:0}$, *iso*-$C_{15:0}$ and *anteiso*-$C_{17:0}$. The genomic DNA G+C content of the type strain is 70.8%.

The type strain, MG28^T (= MCCC 1K06895^T = JCM 34922^T) was isolated from rhizosphere soil of *Akebia trifoliat*e, in Changde of Hunan Province, China. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and

Table 3 Comparative genotypic analysis between strain MG28^T and the closest related type strains, *Streptomyces phaeolivaceus* GY16^T, *Streptomyces europaeiscabiei* KACC 20186^T, *Streptomyces fructofermentans* CGMCC 4.1593^T, *Streptomyces scabiei* NRRL B-16523 and *Streptomyces deccanensis* KCTC 19241^T

Closest related species	Strain MG28^T			
	16S rRNA gene sequence similarity (%)	dDDH (%)	ANIm (%)	ANIb (%)
S. phaeolivaceus GY16^T	99.03	37.3	90.3	88.1
S. europaeiscabiei KACC 20186^T	98.90	38.8	90.7	88.0
S. fructofermentans CGMCC 4.1593^T	98.89	23.2	85.8	80.2
S. scabiei NRRL B-16523^T	98.83	36.1	89.9	87.3
S. deccanensis KCTC 19241^T	98.90	47.0	92.9	91.4

genome sequence of strain MG28^T are OM368590 and CP080647, respectively.

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Author contributions WZ and JG conceived the idea of the study; PM, KL, JZ, FZ, YC, XL, XL, KH, WZ and JG analysed the data; WZ and JG interpreted the results. PM, KL, WZ and JG wrote the paper. All authors discussed the results and revised the manuscript.

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Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

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

Ethical approval This article does not contain any studies with human participants or animal experiments by any of the authors.

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