Streptomyces Pimoensis sp. nov., Isolated From the Taklimakan Desert in Xinjiang, China

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Abstract

A novel *Streptomyces* strain, designated TRM 75549^T, was separated from a sample of sand in Pimo, Taklimakan desert, Xinjiang, North-West China. Phylogenetic analyses of the 16S rRNA gene sequences placed strain TRM75549^T within the genus *Streptomyces* with the highest similarities to *Streptomyces flavoviridis* NBRC 12772^T (98.76%). The whole-genome average nucleotide identity (ANI) value between strain TRM75549^T and *S. flavoviridis* NBRC 12772^T is 88.20%. Digital DNA-DNA hybridization (dDDH) value between strain TRM75549^T and *S. flavoviridis* NBRC 12772^T is 44.10%. They are well below the recommended 95-96% and 70% cut-off points for designated species respectively. A multi-locus sequence analysis of five house-keeping genes (*atpD*, *gyrB*, *recA*, *rpoB* and *trpB*) and phylogenomic analysis also illustrated that strain TRM75549^T should be assigned to the genus *Streptomyces*. Strain TRM75549^T contained MK-9 (H_6) and MK-9 (H_8) as predominant menaquinones. The diagnostic diamino acid of cell walls was identified as LL-diaminopimelic acid and Meso-diaminopimelic. The whole-cell sugar pattern of strain TRM 75549^T consisted of mannose and glucose. The major fatty acids (>5%) were iso-$C_{14:0}$, iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{16:1}$H, iso-$C_{16:0}$. The polar lipids were diphosphatidylglycerol, lysophosphatidylglycerol, phosphatidylethanolamine, phospholipids, phosphatidylglycerol, phosphatidylinositol, phosphatiylinositol mannosides and unidentified phospholipids. Strain TRM75549^T could be differentiated from *S. flavoviridis* NBRC 12772^T, based on physiological and biochemical characteristics. Based on the data from this polyphasic study presented above, strain TRM75549^T is represent ative of a novel species of the genus Streptomyces, for which the name *Streptomyces pimoensis* sp. nov. is proposed. The type strain is TRM75549^T (=CCTCC AA 2020054^T=LMG 32221^T).

Introduction

The genus *Streptomyces* was first proposed by WaKsman and Henrici (1943). *Streptomyces* species are aerobic actinomycetes with a GC-rich genome, most of which can form broadly branched matrix mycelia and aerial mycelia, which usually differentiate into spore chains (Gadagkar and Kumar 2005). Many members of the genus *Streptomyces* are known to produce a variety of biologically active metabolites, including antibiotics, enzymes, enzyme inhibitors, vitamins, etc. (Liu et al. 2018).

In recent years, as domestic and foreign scholars have continuously explored the active products of *Streptomyces*, the research of new species of *Streptomyces* has gradually become a new direction. The isolation sources of new species of *Streptomyces* also include various special environments such as oceans, deserts, and animal and plant bodies.

In this article, the strain TRM75549^T was separated from a sample of sand in Pimo, Taklimakan desert, Xinjiang, North-West China. It has good antibacterial activity against the three pathogenic bacteria gram-positive bacteria *Staphylococcus aureus* ATCC25923, *Enterococcus faecalis* ATCC29212 and gram-negative bacteria *Escherichia coli* ATCC25922. Therefore, this strain was selected for heterogeneous classification and identification. In the present study, the taxonomic status of strain TRM75549^T was

determined using a polyphasic approach. The resultant data showed that TRM75549^T represents a novel *Streptomyces* species for which the name *Streptomyces pimoensis* is proposed.

Materials And Methods

Strain isolation and culturing

The strain TRM75549^T was isolated by standard dilution plate method by growth on Gauze's No. 1 medium (g/L): soluble starch 20 g, KNO_3 1 g, K_2HPO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, NaCl 20 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, Agar 16 g, pH7.0–7.6. (Guan et al. 2010), incubated at 30 ℃ for 10 days, and was preserved in 20% (V/V) glycerol at –20 ℃ and lyophilized in 20% skim milk powder. Biomass for chemical and molecular research was obtained by cultured the strain in trypsin soy broth for 14 days on oscillators at 180 rpm and 30 ℃. The closely related type strains *Streptomyces flavoviridis* DSM 40153 was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) and was cultivated under comparable conditions as reference strain.

Morphological, cultural, physiological, and biochemical characteristic

The cultural characteristics of strain TRM75549^T were determined on ISP media (ISP1, ISP2, ISP3, ISP4, ISP5, ISP6 and ISP7)(Shirling EB, Gottlieb D 1966), Czapek's agar, Potato dextrose agar, Gauze's No. 1 medium and Nutrient agar (Waksman 1967) for 14 days at 30°C. Morphology of mycelia and spores of strain TRM75549^T were observed by optical microscopy and scanning electron microscopy as described by Williams and Davies(1967) after 10 daysof incubation at 30°C on Gauze's No. 1 medium.

Different temperatures (4, 10, 15, 28, 30, 37, 40, 45, 50 and 55 degrees C), pH (pH 4.0-12.0, interval 1.0 pH unit) (Xu et al., 2005) and NaCl concentrations as described in Goodfellow (1986), measured 0-10% on Gauze's No. 1 medium at intervals of 1%, w/v). Physiological characteristics and utilization of carbon sources were assessed following Williams et al. (1989). The uses of liquefaction of gelatin, milk peptonization and solidification, urea, starch hydrolysis, nitrate reduction, melanin production and production of H_2S were studied(Gordon 1974; Yokota et al. 1993).

Antibacterial and antifungal activity

The antimicrobial efficacy of these isolates was tested been tested against various organisms *S.aureus* ATCC25923, *E. faecalis* ATCC29212, *E.coli* ATCC25922 using Kirby-Baur agar diffusion method (Maiti et al. 2020). In brief, lawns of test organisms were prepared on agar medium and 14-days-old colonies were placed on the lawn. The plates were kept at 4°C for 2 h for a homogenous distribution of antimicrobial compound before the growth of test organisms followed by the incubation at 37°C for 24 h. After incubation, the zone of inhibitions around the colony was observed and measured.

Chemotaxonomy

The cells collected by centrifugation were washed with distilled water, and then freeze-dried. The method proposed by Staneck and Roberts was used to determine cell wall amino acids(Staneck and Roberts 1974). Use the method proposed by Tang et al. to analyze whole cell sugars. (2009). Polar lipids were extracted and separated by two-dimensional TLC and identified by the method proposed by Minnikin et al. (1984). Menaquinones were extracted from freeze-dried biomass according to the method proposed by Collins et al. (1977), and subjected to HPLC analysis (Wang et al. 2011). According to the method proposed by Sasser (1990), cellular fatty acids were extracted from fresh cells, and GS chromatographic analysis was performed.

Genome sequencing and phylogenetic analysis

The method of Chun and Goodfellow (1995) was used to extract genomic DNA and PCR amplification of 16S rRNA gene sequence. EzBioCloud (https://www.ezbiocloud.net/identify, Yoon et al. 2017) calculated the similarity of the 16S rRNA gene sequence with other strains, and then the sequence of the strains with a close relationship were selected to construct the phylogenetic tree. These sequences were aligned using MEGA 7.0 software (Kumar et al., 2016) with Neighbor-Joining (Saitou 1987), Maximum likelihood (Felsenstein 1981), and Maximum-Parsimony (Fitch 1971) methods to construct phylogenetic trees. The topologies of the phylogenetic trees were evaluated by the bootstrap resampling method with 1000 replicates (Felsenstein 1985). A complete genome sequence of strain was obtained using an Illumina platform and assemble by Velvet 1.2.10. The G+C content of genomic DNA was determined from the whole genome sequence by Average Nucleotide Identity (ANI) calculator (http://www.ezbiocloud.net/tools/ani). The digital DNA-DNA hybridization (dDDH) values were calculated on the GGDC website using formula 2(http://ggdc.dsmz.de/ggdc.php), originally described by Auch et al (2010) and updated by Meier-Kolthoff et al (2013). The genome sequences of strain TRM75549^T (accession no. JAHWZY000000000) and strain *S. flavoviridis* NBRC 12772^T (accession no. BMTE00000000) have been submitted to GenBank. Housekeeping genes used for multilocus sequence analysis (MLSA), as used in previous *Streptomyces* analysis, were *atp*D (ATP synthase subunit D), *gyr*B (DNA gyrase B subunit), *rec*A (recombinase A), *rpo*B (RNA polymerase beta subunit) and *trp*B (tryptophane B, beta subunit). Each locus for each strain was concatenated head to tail in frame as follows: *atp*D, *gyr*B, *rec*A, *rop*B and *trp*B. The sequences for all loci of other related strains were obtained from the NCBI (https://www.ncbi.nlm.nih.gov/).

Results And Discussion

After the strain TRM75549^T was cultured at 30°C for 7 days, it was grown on 11 standard media, and the colors of aerial mycelia and substrate mycelia were recorded (Table 1). The strain was observed to grow well on ISP 1, ISP 4, ISP 5, ISP 7, Gauze's No. 1 agar, moderately well on ISP 2, Czapek's agar, Patato dextrose agar, with slow growth on ISP 3 and ISP 6, and none on nutrient agar. The results of the antibacterial experiment showed that TRM75549^T inhibits *E. coli* with an average diameter of 2 cm, inhibits *E. faecalis* with an average diameter of 1.5 cm, and inhibits *S. aureus* with an average diameter of

1.3 cm; *Streptomyces flavoviridis* DSM 40153^T inhibits E.coli with an average diameter of 1.1cm, and inhibits *E. faecalis*, with an average diameter of 1.3 cm, but *S.aureus* has no obvious transparent circle (Supplementary Fig. S1). Strain TRM75549^T could be distinguished from *S. flavoviridis* DSM 40153^T by some phenotypic characteristics, inparticular cultural characteristics (Supplementary Table S1). Morphological characteristics of strain TRM75549^T were observed using SEM (Fig. 1). The strain was observed to form an abundant white aerial mycelium, occasionally twisted, which differentiates into spore chains. Each spore was observed to be olivary with a hairy surface. Strains grow at 15-45°C, pH6.0-9.0 and 0-9% NaCl, and are best grown at 30°C and pH 7.0 at 1% (w/v) NaCl. The strains of hydrogen peroxide enzymes, amyloid hydrolysis, nitrate reduction, cellulose decomposition were all positive, while urease, gelatin liquefaction, milk peptonization and solidification, oxidase, melanin production, hydrogen sulfide production were negative. The strain could degrade Tweens 20, 40, 60 and 80.

Table 1

Characteristics of strain TRM 75549^T grown on various media at 30 °C for 14 days

Medium	**Growth**	**Colour of mycelia**	
		Aerial	**Substrate**
ISP 1	Good	Grayish white	Pale yellow
ISP 2	Moderate	Grayish white	white
ISP 3	Poor	pale green	kelly green
ISP 4	Good	Grayish white	Pale yellow
ISP 5	Good	Yellowish white	kelly green
ISP 6	Poor	Grayish white	Greyish yellow
ISP 7	Good	Grayish white	Greyish yellow
Gauze's No. 1 agar	Good	Grayish white	kelly green
Czapek's agar	Moderate	Grayish white	white
Patato dextrose agar	Moderate	Grayish white	white
nutrient agar	None	None	None

The cell wall of strain TRM75549^T contained LL-diaminopimelic acid and Meso-diaminopimelic, and the whole cell hydrolyzed sugar type was mannose and glucose. The polar lipids were diphosphatidylglyceroln (DPG), lysophosphatidylglycerol (LPG), phosphatidylethanolamine (PE), phospholipids (PLS), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylinositol mannosides (PIM) and an unidentified phospholipid (Supplementary Fig.S2). The predominant

menaquinones were MK-9 (H_6) and MK-9 (H_8). The major fatty acids (>5%) were iso-$C_{14:0}$ (10.64%), iso-$C_{15:0}$ (5.01%), anteiso-$C_{15:0}$ (11.98%), iso-$C_{16:1}$ H (12.87%), iso-$C_{16:0}$ (33.46%).

The GenBank login number of the 16S rRNA gene sequence of the strain TRM75549^T is MW479154, and the closest phylogenetic neighbor was *S. flavoviridis* NBRC 12772^T (GenBank accession no. AB184842). The average nucleotide identity value and the digital DNA-DNA hybridization value between strain TRM75549^T and *S. flavoviridis* NBRC 12772^T were 88.20% and 44.10%, respectively, well below 95–96% and 70% cut-off point recommended for delineating species. The phylogenetic tree constructed from the 16S rRNA gene sequence through the Neighbor-Joining method showed that the strain TRM75549^T formed a unique clade (Fig. 2), which was also restored in the Maximum-Likelihood trees and Maximum-Parsimony trees (Supplementary Fig. S3, S4). The MLSA with the concatenated *atpD*, *gyrB*, *recA*, *rpoB* and *trpB* genes showed that the strain is clustered with *S. flavoviridis* NBRC 12772^T, and the MLSA distances were much greater than the generally accepted threshold value of 0.007 for species delineation (Fig. 3). The G+C content in the draft genome sequence of strain TRM 75549^T was identified as 72.14 mol%. All the data suggested that strain TRM75549^T was a member of the genus *Streptomyces*. However, on the basis of a combining compare of phylogenetic distinctness and differences in chemotaxonomic and physiological characteristics (Table 2), it is considered that strain TRM75549^T is the representative of a new species of *Streptomyces*, named *Streptomyces Pimoensis* sp. nov. is proposed. The type strain is TRM 75549^T (=CCTCC AA 2020054^T=LMG 32221^T).

Table 2

Biochemical characteristics of strain TRM 75549^T compared to its phylogenetic relatives

Characteristic	**1**	**2**
Spore-chain morphology	Straight	Spirale
Spore surface	Hairy	Spiny
Optimum temperature	30℃	28℃
pH tolerance	6.0–9.0	7.0–9.0
NaCl tolerance (%,w/v)	0–9	0–5
Glucose	+	+
L(+) Arabinose	+	+
Cellobiose	+	+
D(+)Fructose	+	+
D(+)Galactose	+	+
D(+)Maltose	+	+
D(+)Mannitol	+	+
Lactose	+	-
Xylose	+	+
Raffinose	-	-
myo-Inositol	w	+
Sucrose	w	-
L-Rhamnose	-	+
Gelatin liquefaction	+	w
Starch hydrolysis	+	+
Nitrate reductase	+	+
Urea	-	-
H_2S production	-	+
Melanin production	-	-
Milk peptonization	-	+
Major whole-cell sugars	Glucose	Glucose

	mannose	
Major cell-wall diamino acid	LL-DAP meso-DAP	LL-DAP
Phospholipids	DPG, PE, PI, PG, PIM, PLS, LPG	DPG, PE, PC, PG, NPG, PLS, LPG
Predominant menaquinones	MK-9(H_6), MK-9(H_8)	MK-8,MK-8(H_2) MK-8(H_6), MK-9(H_6)
Major cellularfatty acids (>10%)	iso-$C_{16:0}$, iso-$C_{16:1}{}^{H}$, anteiso-$C_{15:0}$, iso-$C_{15:0}$	iso-$C_{16:0}$, anteiso-$C_{15:0}$, $C_{16:0}$, Sum 3

Strains: 1, TRM75549$^{T;}$ 2, *Streptomyces flavoviridis* DSM 40153^{T}; All data are from this study. + , Positive; w, weakly positive; -, negative.

Description of Streptomyces pimoensis **sp. nov.**

Streptomyces pimoensis (pi.mo.en'sis N.L.Masc. adj. pimoensis. Pertaining to Pimo, Taklimakan desert, Xinjiang, North-West China, from where the type strain was isolated).

This species is aerobic, Gram-positive actinomycetes. The aerial mycelium is densely straight or tortuous, and each spore was observed to be olivary with a hairy surface. Grow well on ISP 1, ISP 4, ISP 5, ISP 7, Gauze's No. 1 agar, grow moderately on ISP 2, Czapek's agar, Patato dextrose agar. The strain can grow at 0–9% (w/v) NaCl, pH 6.0–9.0 and 15–45°C, with optimum growth at 1% (w/v) NaCl, pH 7.0–8.0, and 30°C respectively. Glucose, arabinose, D-sorbitol, D-xylose, fructose, maltose, mannitol, lactose, D-galactose, inositol, ribose, cellobiose are utilized. Sucrose, rhamnose, raffinose, trehalose are not utilized. Starch hydrolysis, nitrate reduction, cellulose decomposition, degradations of Tweens 20, 40, 60 and 80 are positive, whereas urease, gelatin liquefaction, milk peptonization and solidification, oxidase, melanin production, H_2S production are negative. The cell wall contains LL-diaminopimelic acid and meso-diaminopimelic. Whole cell hydrolysates contain mannose and glucose. The polar lipids were diphosphatidylglyceroln (DPG), lysophosphatidylglycerol (LPG), phosphatidylethanolamine (PE), phospholipids (PLS), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatiylinositol mannosides (PIM) and an unidentified phospholipid. The menaquinone system contains MK-9 (H_6) and MK-9 (H_8). The major fatty acids are iso-$C_{14:0}$ (10.64%), iso-$C_{15:0}$ (5.01%), anteiso-$C_{15:0}$ (11.98%), iso-$C_{16:1}$H (12.87%), iso-$C_{16:0}$ (33.46%).

The type strain is TRM75549^{T} (=CCTCC AA 2020054^{T}=LMG 32221^{T}), isolated from the Pimo, Taklimakan desert, Xinjiang, North-West China. The G+C content in the draft genome sequence of strain TRM75549^{T} is 72.14 mol%. The GenBank/EMBL/DDBJ accession number for the genome and 16S rRNA gene sequence of strain TRM75549^{T} is JAHWZY000000000 and MW479154.

Declarations

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Author contributions

Ping Zhang participated in the experiment and preparation of the first draft. Xiaoxia Luo,Xinrong Luo,Zhanwen Liu, Zhanfeng Xia, and Chuanxing Wan gave guidance during the experiment. Lili Zhang contributed to reagents, instrumentation and the financial support for this work.

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Compliance with ethical standards

The authors state that there is no confict of interest.

Ethical approval

This article does not contain any research conducted by any author on human participants or animals.

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Figures

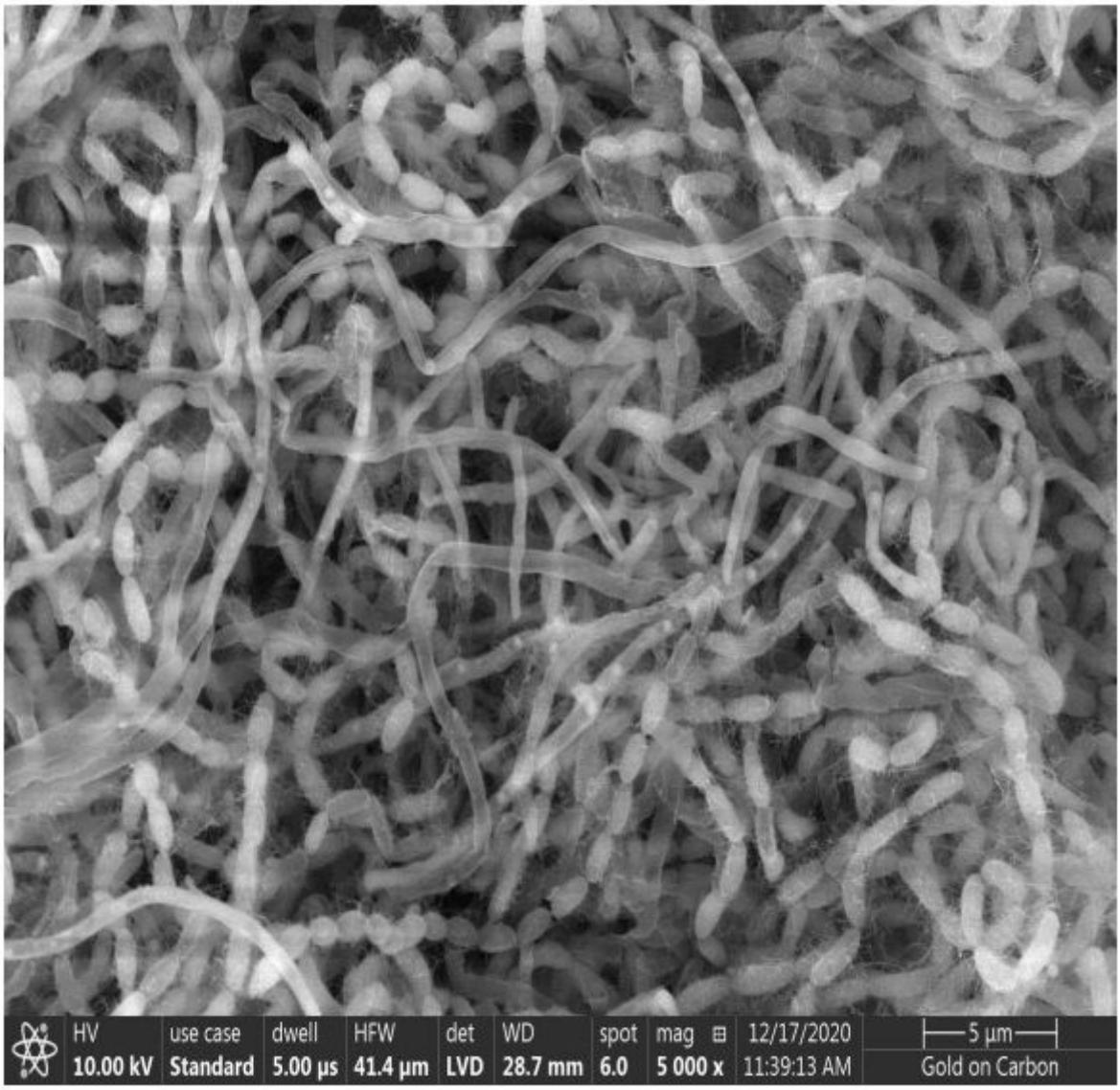

Figure 1

Scanning electron microscopy image of strain TRM75549T grown on Gauze's No. 1 agar at 30 °C for 7 days. Bars, 5 µm.

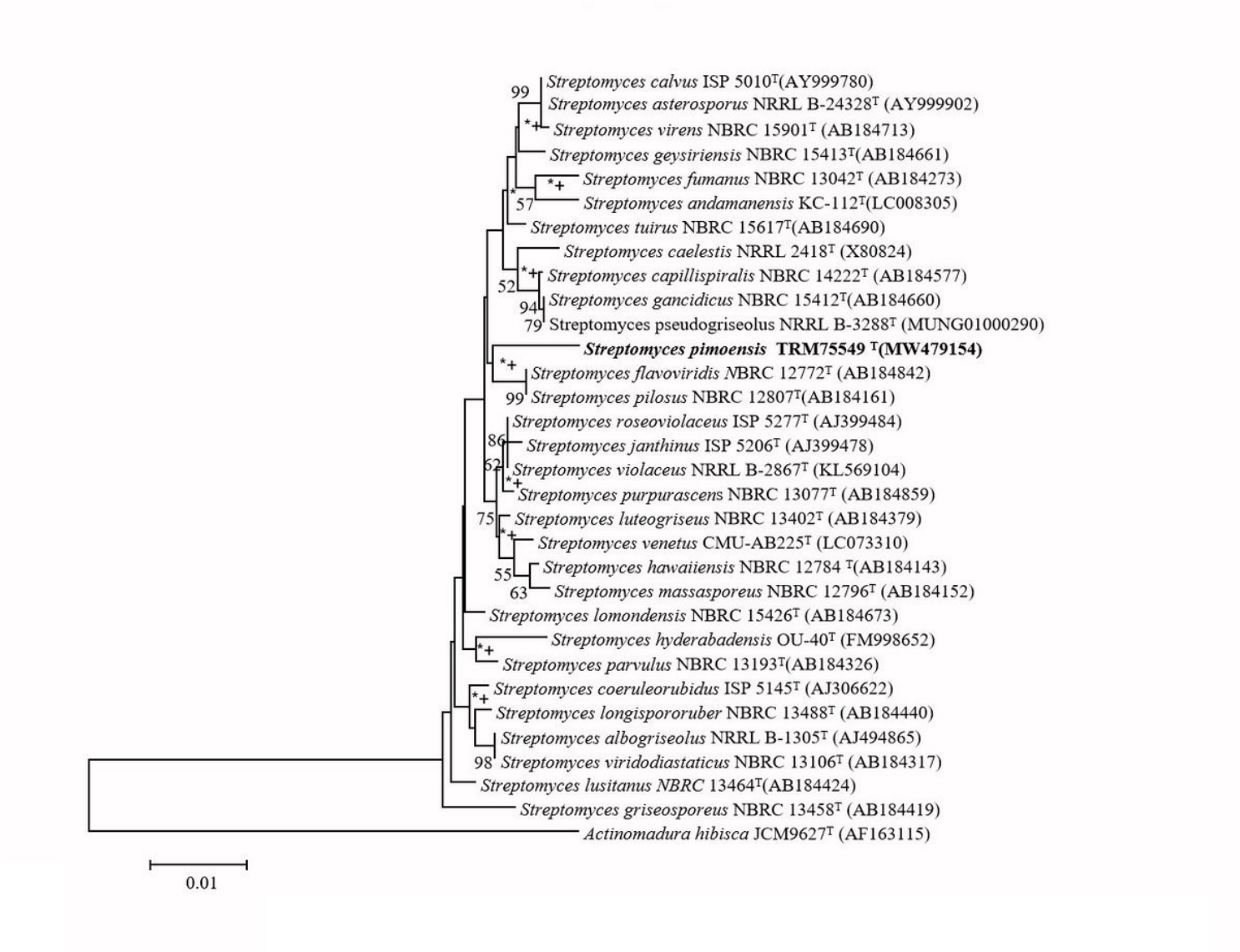

Figure 2

Neighbour-joining unrooted tree based on 16S rRNA gene sequences, illustrating the positions of strain TRM75549Tand related taxa. *, Branches that were also found using the maximum-parsimony method; +, branches that were also found using the maximum-likelihood method; *+, Branches that were found using all three methods. Numbers at nodes are percentage bootstrap values based on 1000 resampled datasets; only values above 50% are indicated. Bar, 0.01 substitutions per nucleotide position.

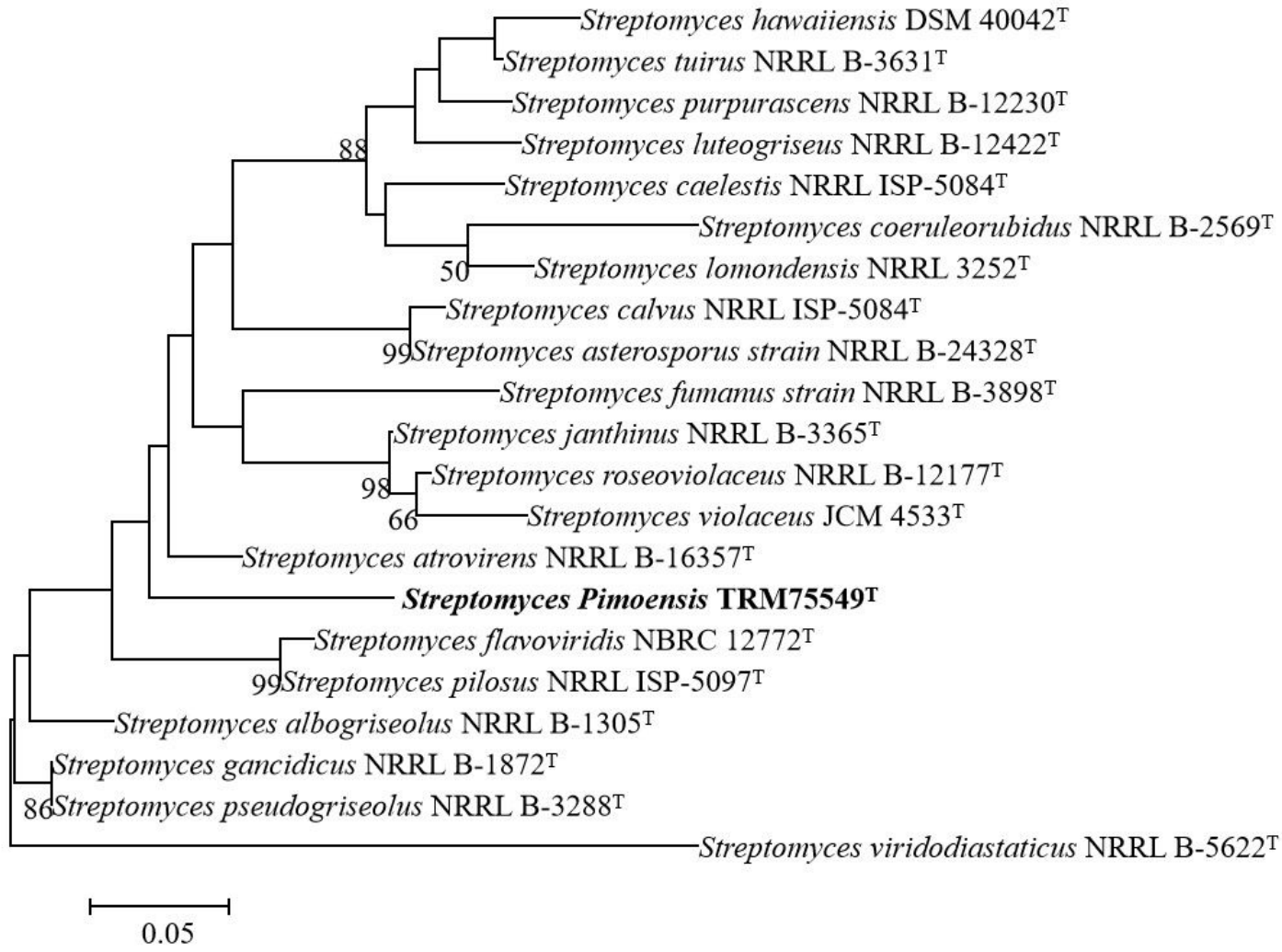

Figure 3

Neighbour-joining unrooted tree based on concatenated partial sequences of five housekeeping genes (atpD, gyrB, recA, rpoB and trpB) showing the position of strain TRM75549Tamongst its phylogenetic neighbours. Only bootstrap values above 50% (percentages of 1000 replications) are indicated. Bar, 0.05 substitutions per nucleotide position.

Supplementary Files

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