Streptomyces Grisecoloratus Sp. Nov., a New Bacterium Isolated From Soil in Cotton Fields in Xinjiang, China

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Original Paper

Keywords: Streptomyces grisecoloratus, Streptomycetaceae, novel species, polyphasic, taxonomy

Posted Date: February 16th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-201561/v1

Abstract

A novel bacterium of the *Streptomyces* genus, designated TRM S81-3^T, was isolated from soil in cotton fields of Xinjiang, China. Comparative 16S rRNA gene sequence analysis indicated that strain TRM S81-3^T ismost closely related to *Streptomyces naganishii* NBRC 12892^T (98.96% sequence similarity); however, the average nucleotide identity (ANI) between strains TRM S81-3^T and *S. naganishii* NBRC 12892^T is relatively low (86.26%). Strain TRM S81-3^T possesses LL-diaminopimelic acid as the diagnostic cell-wall diamino acid, MK-9(H_4), MK-9(H_6), and MK-9(H_{10}) as the major menaquinones, and polar lipids including DPG, PE, PC, PI, PME, NPG and PL. The major fatty acids are *iso*-$C_{16:0}$, *anteiso*-$C_{15:0}$, *anteiso*-$C_{17:1}$ *ω9c*, *anteiso*-$C_{17:0}$, *iso*-$C_{15:0}$, and $C_{14:0}$. The genomic DNA G+C content is 72.1%. Based on the evidence from this polyphasic study, strain TRM S81-3^T represents a novel species of *Streptomyces*, for which the name *Streptomyces grisecoloratus* is proposed. The type strain is TRM S81-3^T (=CCTCC AA 2020002^T=LMG 31942^T).

Introduction

The genus *Streptomyces*, first proposed by Waksman and Henrici (Waksman and Henrici 1943), belongs to the family Streptomycetaceae. At the time of writing, more than 957 species of *Streptomyces* have been described (Genus: *Streptomyces* (bacterio.net)). *Streptomyces* strains are widely distributed and found in a variety of environments, including the desert (Li et al. 2019), sediments (Ay et al. 2018, Hu et al. 2012), insects (Ye et al. 2017), lichens (Saeng-In et al. 2017), the rhizosphere (Piao et al. 2018), and plants (Wang et al. 2018). Members of the genus *Streptomyces* are Gram-positive, aerobic actinomycetes that have high DNA G + C contents (69–73 mol%) (Manfio 1995, Anderson and Wellington 2001). These species have diverse metabolic pathways and potential applications in the production of antibiotics, vitamins, enzymes, enzyme inhibitors, and bioactive compounds of importance to the food, agricultural, and pharmaceutical industries (Lazzarini et al. 2000, McCarthy and Williams 1992). In this study, we isolated an actinomycete strain, designated TRM S81-3^T. We performed a polyphasic taxonomic analysis of this strain and propose that it represents a novel species of the genus *Streptomyces*.

Materials And Methods

Strain isolation and culturing

Strain TRM S81-3^T was isolated from a soil sample collected from cotton fields in Xinjiang in northwest China (40°22'N 80°30'E). The sample was isolated on GJ medium using a 10-fold dilution series method with incubation at 28°C. The composition of GJ medium was (per liter of distilled water): 2 g arginine, 12.5 g glycerin, 0.01 g $FeSO_4 \cdot 7H_2O$, 2 g $K_2HPO_4 \cdot 3H_2O$, and 16 g agar. The strain was purified on Gause's medium at 28°C. The strain was stored in 20% glycerol for short-term storage and lyophilized in 20% skim milk powder for long-term storage.

Morphological, culture, physiological, and biochemical characteristics

To determine the culture characteristics, strain TRM S81-3^T was cultured on a series of ISP media (ISP1, ISP2, ISP3, ISP4, ISP5, ISP6, and ISP7) (Shirling and Gottlieb 1966), Gause's synthetic medium (Atlas 1993), Czapek's agar, potato dextrose agar, and nutrient agar medium (Waksman 1967). The medium was adjusted to pH 7.0–7.5. The organism was grown and maintained on Gause's synthetic medium. Cell morphological observations of spores and mycelia were conducted by SEM (JSM-6360; JEOL, Ltd., Tokyo, Japan) of cultures on Gause's synthetic plates incubated at 28°C for 1 week. Carbon-source utilization tests were performed according to the method described by Shirling et al. (1966) and using the basal medium recommended by Pridham and Gottlieb (1968). The ability of strain TRM S81-3^T to grow from 10°C–55°C (10°C, 12°C, 15°C, 20°C, 25°C, 28°C, 30°C, 37°C, 40°C, 45°C, 50°C, and 55°C) and pH 4–12 (pH 4, 5, 6, 7, 8, 9, 10, 11, and 12) and to tolerate concentrations of 0%–10% (0%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, and 10%, w/v) NaCl was tested using Gause's agar as the basal medium. The production of peroxidase, urease, esterase, and catalase was tested using the method described by Gerhardt et al. (1994) . The use of a sole carbon source (0.5%, w/v), cellulose decomposition, starch hydrolysis, liquefaction of gelatin, milk peptonization and solidification, nitrate reduction, and production of H_2S (Gordon 1974, Yokota et al. 1993) were studied.

Chemotaxonomy

Biomass used for studies was obtained by culturation in liquid Gause's medium for 7 days with shaking at 28°C. Standard procedures were used to determine the type of amino acids and sugars in cell-wall hydrolysates (Hasegawa et al. 1983). Menaquinones were extracted using the method of Collins (1985) and analyzed by HPLC (Groth et al. 1997). Polar lipids were extracted, examined by two-dimensional TLC, and identified by using the procedures of Minnikin et al. (1984). Cellular fatty acid composition was determined as described by Kampfer et al. (1996) using the Sherlock Microbial Identification System (Version 6.1; MIDI database: RTSBA6; MIDI Inc., Newark, DE, USA).

Genome sequencing and phylogenetic analysis

Genomic DNA of strain TRM S81-3^T was extracted from cells grown on Gause's liquid medium for a week at 28°C and used as a template for subsequent PCR amplification. Amplification and sequencing of the 16S rRNA gene were performed as described by Kimc et al. (2000). Alignments of multiple 16S rRNA sequences of closely related members of the genus *Streptomyces* and sequence similarity calculations were carried out using the EzTaxon-e server (Yoon et al. 2017.). Multi-locus sequence analysis (MLSA) was carried out using the housekeeping genes used in previous *Streptomyces* analyses: *atpD* (ATP synthase F1, beta subunit), *gyrB* (DNA gyrase B subunit), *recA* (recombinase A), *rpoB* (RNA polymerase, beta subunit), and *trpB* (tryptophan synthase, beta subunit). Sequences of strain TRM S81-3^T were obtained through genome sequencing (GenBank number: JACVQF000000000), and the position of these genes in the genome are 01437, 02082, 01122, 03486, and 01287, respectively. The sequences for these loci in related strains were obtained from the ARS Microbial Genome Sequence Database server

(http://199.133.98.43). For each strain, these five loci were concatenated head-to-tail in-frame as follows: *atpD*, *gyrB*, *recA*, *rpoB*, and *trpB*. Phylogenetic trees for 16S rRNA and the concatenated multi-locus sequences were constructed using the neighbor-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981), and maximum-parsimony (Mount 2008) algorithms in the MEGA X program (Kumar et al. 2018). For the neighbor-joining method, evolutionary distance matrices were calculated using the Kimura two-parameter model (Kimura 1980) with SeaView, Version 4.2 (Gouy et al. 2010). For maximum-likelihood analysis, the best model (JTT+I+G) was chosen via the program ProtTest 3 (Darriba et al. 2011). The topologies of the resultant phylogenetic trees were evaluated by bootstrap resampling with 1000 replicates (Felsenstein 1992). Because the topologies of these trees were similar, only the neighbor-joining tree is shown (Fig. 2). To determine genomic relatedness, the average nucleotide identity (ANI) was determined using OrthoANI with default parameters (https://www.ezbiocloud.net/tools/ani) (Yoon et al. 2017).

Results And Discussion

After one week of culture, strain TRM S81-3^T exhibited branched substrate hyphae and aerial hyphae. Under the electron microscope, the spore surface was spiny, and the spores were short and rod-shaped with dimensions of approximately 0.5 μm×1.5 μm (Fig. 1). Culture characteristics of strain TRM S81-3^T were determined on ISP series media (ISP1, ISP2, ISP3, ISP4, ISP5, ISP6, and ISP7), Gause's synthetic medium, Czapek's agar, potato dextrose agar, and nutrient agar medium. Poor growth was observed on ISP5 medium and Czapek's agar, and aerial hyphae did not form on ISP6 medium. The growth on other media was vigorous. No diffusible pigments or melanin were observed on any of the media tested. The growth ranges of TRM S81-3^T were temperatures 10°C–50°C and pH 4.0–12.0, with optimal growth at 28°C and pH 5.0–10.0. The NaCl concentration range for growth was 0–10%, with optimal growth at 0%. Other physiological characteristics of strain TRM S81-3^T are given in the species descriptions (Table 1). The strain was positive for nitrate reduction, catalase production, milk coagulation and peptization, starch hydrolysis, cellulose hydrolysis, and urease production but negative for gelatin hydrolysis, oxidase production, melanin production, and H_2S production. The strain could degrade Tweens 20, 40, 60, and 80.

Strain TRM S81-3^T contained LL-diaminopimelic acid as its cell-wall diamino acid, and whole-cell hydrolysates contained mainly ribose, xylose, and mannose. The predominant menaquinones of strain TRM S81-3^T were MK-9(H_4), MK-9(H_6), and MK-9(H_{10}). The major cellular fatty acids were *iso*-$C_{16:0}$ (42.06%), *anteiso*-$C_{15:0}$ (11.90%), *anteiso*-$C_{17:1}$ *ω*9*c* (9.19%), *anteiso*-$C_{17:0}$ (7.21%), *iso*-$C_{15:0}$ (6.94%) and $C_{14:0}$ (5.93%). Polar lipids consisted of diphosphatidylglycerol (DPG), phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl methyl ethanolamine (PME), one phospholipid of unknown structure containing glucosamine (NPG), and two unidentified phospholipids (PLs, Online Resource 1).

Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain TRM S81-3^T falls within the genus *Streptomyces* and had the highest sequence similarity (98.96%) to *Streptomyces naganishii*

NBRC 12892^T (GenBank accession no. AB184224). The analysis placed TRM S81-3^T in a clade with *S. naganishii* NBRC 12892^T, and its neighbors were two other *Streptomyces* strains, *S. ruber* NBRC 14600^T and *S. roseiscleroticus* NBRC 13002^T. All four species sit on the same branch, and TRM S81-3^T shares 16S rRNA similarities of 98.37% and 98.27% with *S. ruber* NBRC 14600^T and *S. roseiscleroticus* NBRC 13002^T, respectively. The phylogenetic analysis also showed that strain TRM S81-3^T forms a distinct clade from other closely related species of the genus *Streptomyces* (Fig. 2). The topologies of phylogenetic trees built using the maximum-likelihood (Online Resource 2) and maximum-parsimony (Online Resource 3) algorithms were similar to that of the neighbor-joining tree. The MLSA phylogenetic analysis shows the near neighbor of TRM S81-3^T is *S. viridiviolaceus* NBRC 13359^T (MLSA distance = 0.0206) (Fig. 3), while the MLSA distances were much greater than the generally accepted threshold (> 0.007) for species delineation using this scheme (Rong and Huang 2012). The topologies of phylogenetic trees built using the maximum-likelihood (Online Resource 4) and maximum-parsimony (Online Resource 5) algorithms were similar to that of the neighbor-joining tree. A draft genome sequence was determined for strain TRM S81-3^T. The draft genome size was 8,820,456 bp and comprised 241 contigs. The GC content was 72.1%. Strain TRM S81-3^T and *S. naganishii* NBRC 12892^T showed 86.26% ANI to each other, which is below the threshold of the 95–96% ANI cut-off widely accepted for delineating prokaryotic species (Richter and Rosselló-Móra 2009). Based on differences in phenotypic characteristics and the chemotaxonomic and phylogenetic data, strain TRM S81-3^T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces grisecoloratus* is proposed. The type strain is TRM S81-3^T (= CCTCC AA 2020002^T = LMG 31942^T).

Description of Streptomyces grisecoloratus **sp. nov.**

Streptomyces grisecoloratus (gri.se.co.lo.ra'tus. N.L. masc. adj. *griseus* gray; L. masc. past part. *coloratus* colored; N.L. masc. adj. *grisecoloratus* gray-colored).

Aerobic, non-motile, Gram-positive actinomycete that forms an extensively branched substrate mycelium and aerial mycelium that differentiate into straight spore chains with spiny-surfaced spores. The pH and NaCl tolerance ranges for growth are 5.0–10.0 (optimum, pH 7.0) and 0–10% (w/v; optimum, 0% w/v), respectively. The temperature range for growth is between 10°C and 50°C (optimum, 28°C). Poor growth was observed only on ISP5 and Czapek's agar, and it cannot form aerial hyphae on ISP6 medium. Growth on other media is vigorous. No diffusible pigments or melanin were observed on any of the media tested. Uses all carbon sources tested as nutrients, including D-mannitol, D-glucose, L-arabinose, D-fucose, D-xylose, D-fructose, L-rhamnose, D-galactose, D-lactose, D-raffinose, D-inositol, and D-sucrose. Positive for nitrate reduction, catalase production, lipase production, milk coagulation and peptization, starch hydrolysis, cellulose hydrolysis, and urease production but negative for gelatin hydrolysis, oxidase production, melanin production, and H_2S production. The diagnostic phospholipids are DPG, PE, PC, PI, PME, NPG, and PL. Cell-wall sugars are ribose, xylose, and mannose, and the major menaquinones were MK-9(H_4), MK-9(H_6), and MK-9(H_{10}). The major fatty acids are *iso*-$C_{16:0}$, *anteiso*-$C_{15:0}$, *anteiso*-$C_{17:1}$ ω9*c*, *anteiso*-$C_{17:0}$, *iso*-$C_{15:0}$, and $C_{14:0}$. The genomic DNA G + C content of the type strain is 72.1 %.

The type strain TRM S81-3^T (= CCTCC AA 2020002^T = LMG 31942^T) was isolated from cotton fields in Xinjiang, northwest China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TRM S81-3^T is MT756021. The TRM S81-3^T genome sequence was deposited in GenBank (JACVQF000000000).

Declarations

Author contributions L. Xing and Y.-Y. Xia performed the experiments and wrote the initial draft. Q.-Y. Zhang, Z.-F. Xia, C.-X. Wan, and L.-L. Zhang guided the experimental operations. X.-X. Luo contributed reagents, instrumentation, and financial support for this work.

Funding This work was supported by the Program for Young and Middle-Aged Technology Innovation Leading Talents (Project no. 2019CB030), the National Natural Science Foundation of China (Project no. U1703236), the Innovative Team in the Key Areas of the Corps of Microbial Resources (Project no. 2017CB014), and the National Innovative Project for College Students (Project no. 201810757016).

Conflicts of interest The authors declare that there are no conflicts of interest.

Ethical approval No studies with human participants or animals were performed.

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Tables

Table 1. Characteristics of strain TRM S81-3^T compared with its most closely related *Streptomyces* species.

Strains: 1, TRM S81-3^T; 2, *Streptomyces naganishii* NBRC 12892^T (Yamaguchi and Saburi 1955)**; 3, *Streptomyces griseomycini* NBRC 12778^T** (Whitman 2012)**; 4, *Streptomyces griseostramineus* NBRC 12781^T** (Whitman 2012)**.**

+, positive; −, negative; ND, not determined.

Characteristics	1	2	3	4
Spore color	Gray	Brownish-gray	Gray	Gray
Spore wall ornamentation	Spiny	Smooth	Hairy	Spiny
Spore chain morphology	Straight	Spirales	Spirales	Spirales
Growth at 50^{o}C	+	ND	ND	ND
Growth at 10% NaCl	+	-	ND	ND
pH range	5-10	5-11	ND	ND
milk coagulation and peptization	+	+	+	+
Hydrolysis of starch	+	+	+	+
Liquefaction of gelatin	-	+	ND	ND
Carbon source utilization:				
D-Mannitol	+	+	+	+
D-Glucose	+	+	+	+
L-Arabinose	+	+	+	+
D-Fucose	+	+	+	+
D-Xylose	+	+	+	+
D-Fructose	+	+	+	+
L-Rhamnose	+	+	+	+
D-Galactose	+	+	+	+
D-Lactose	+	+	+	+
D-Raffinose	+	-	-	+
D-Inositol	+	+	+	+
D-Sucrose	+	-	-	-

Figures

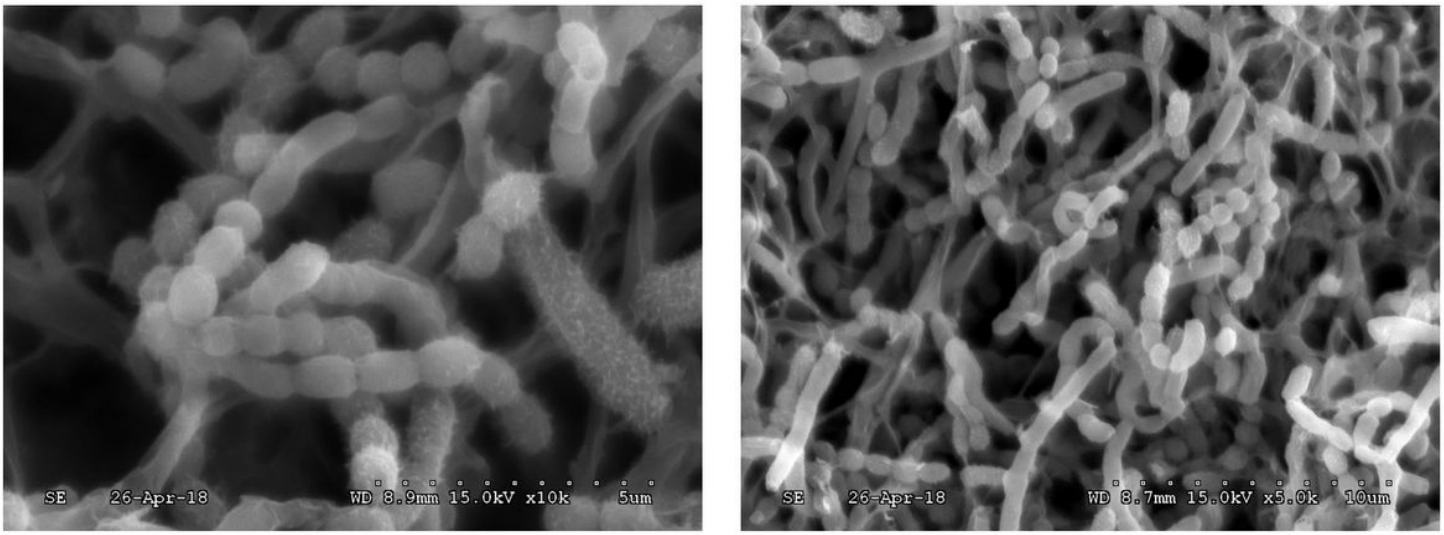

Figure 1

Scanning electron micrograph of strain TRM S81-3T grown on Gause's medium at 28°C for 7 days

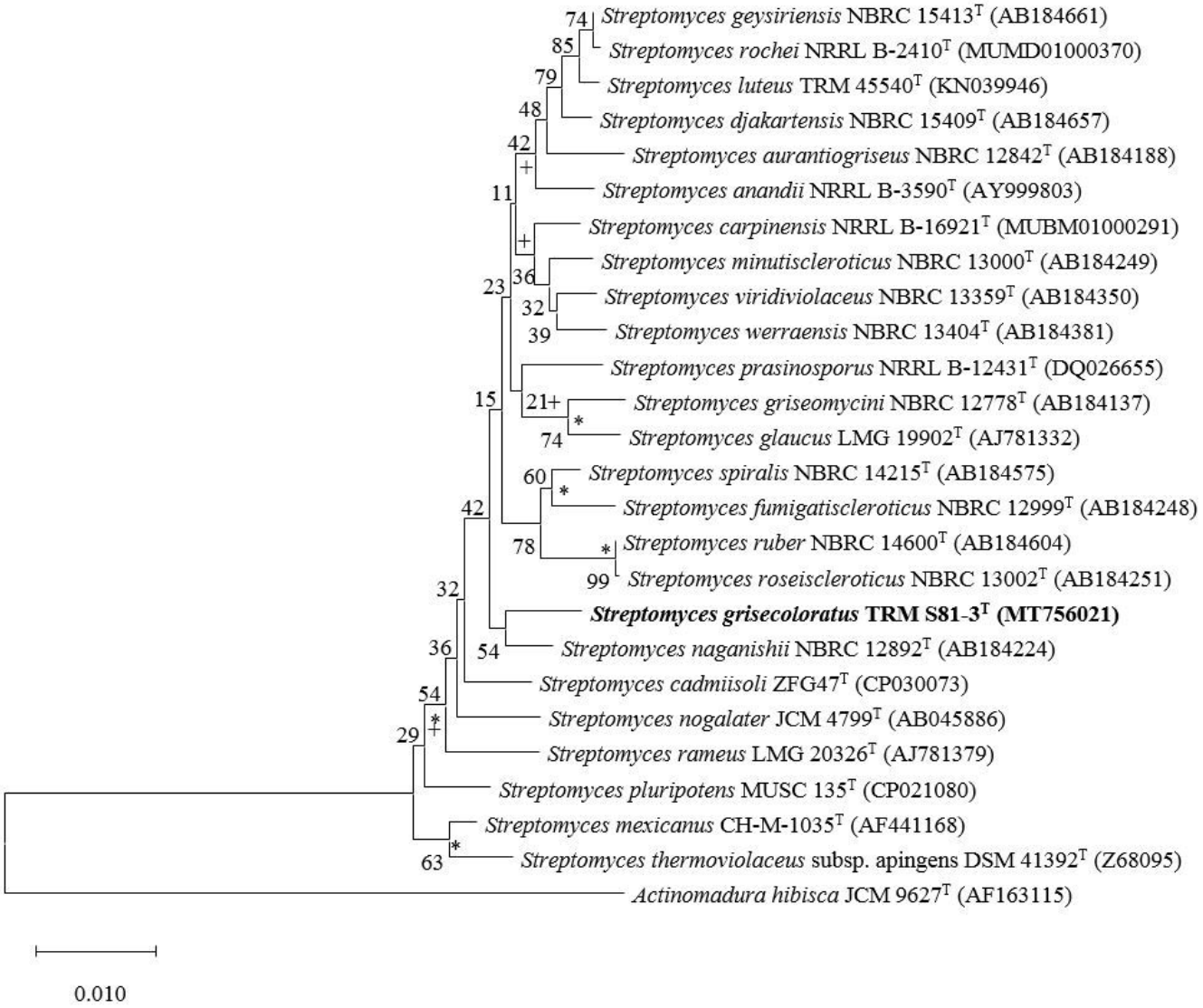

Figure 2

Neighbor-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences The relationships between strain TRM S81-3T and the type strains of closely related Streptomyces species were analyzed. Actinomadura hibisca JCM 9627T (AF163115) was used as the outgroup. *, branches that were also found using the maximum-parsimony method; +, branches that were also found using maximum likelihood; *+, branches that were found using all three methods. Numbers at the nodes are percentage bootstrap values based on 1000 replicates; only values above 50% are given. Bar, 0.0100 substitutions per nucleotide position.

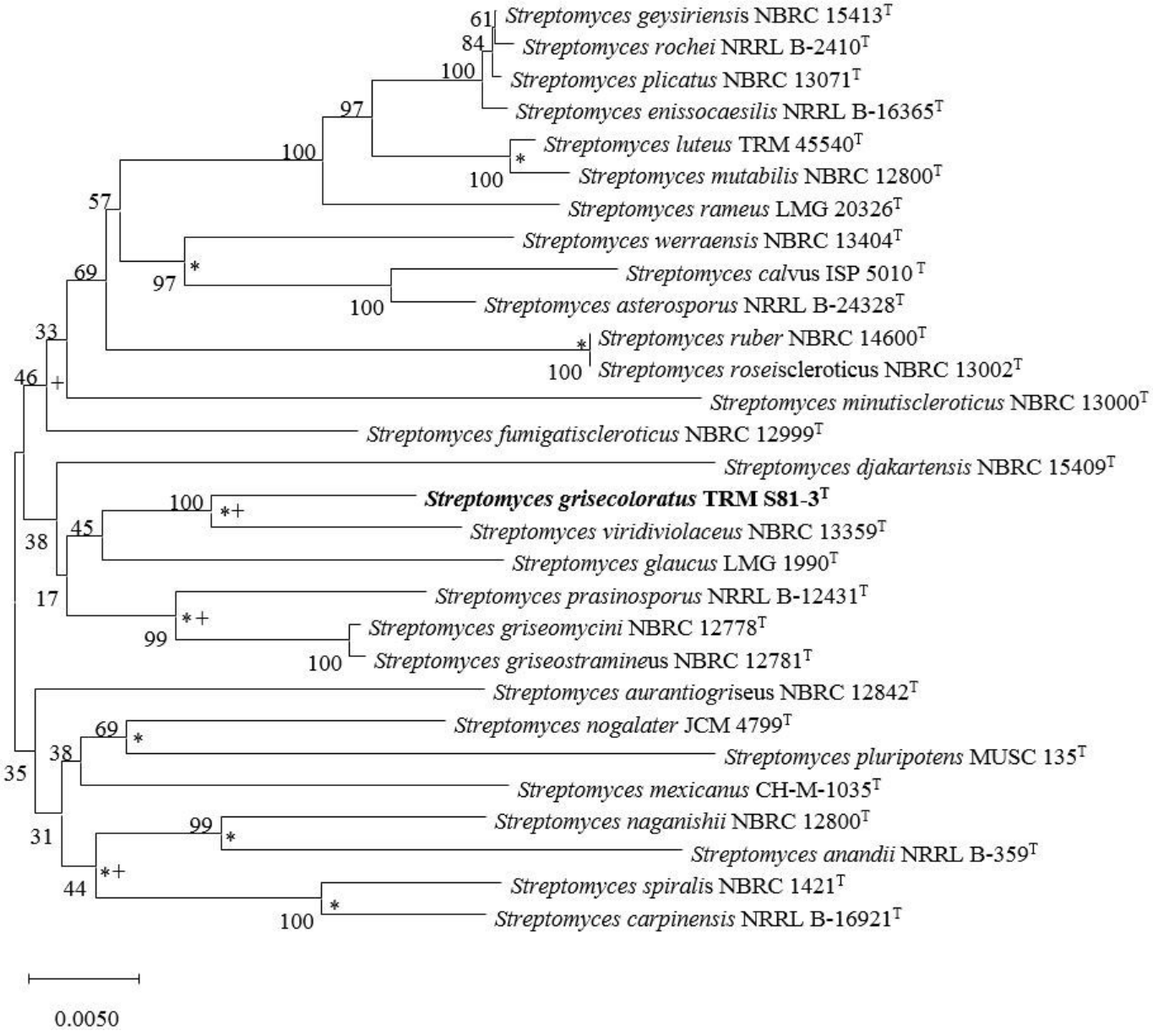

Figure 3

Neighbor-joining phylogenetic tree based on concatenated partial sequences of the housekeeping genes atpD, gyrB, recA, rpoB, and tyrB The relationships between strain TRM S81-3T and the type strains of closely related Streptomyces were analyzed. *, branches that were also found using the maximum-

parsimony method; +, branches that were also found using maximum likelihood; *+, branches that were found using all three methods. Numbers at the nodes are percentage bootstrap values based on 1000 replicates; only values above 50% are given. Bar, 0.0050 substitutions per nucleotide position.

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