Streptomyces qaidamensis sp. nov., isolated from sand in the Qaidam Basin, China

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Abstract

A novel actinobacterial strain, designated S10^T, was isolated from a sand sample collected from the Qaidam Basin in Qinghai province, China. The strain S10^T exhibited antibacterial activity against MRSA. The taxonomic position of the strain S10^T was determined by a polyphasic approach. There were 6 copies of 16S rDNA in S10^T which were not same exactly (MH257693-MH257698). Phylogenetic analysis of 16S rRNA gene sequences indicated the strain belonging to the genus *Streptomyces* and it showed high sequence similarities with *Streptomyces chartreusis* NBRC 12753^T (99.31%), *Streptomyces phaeoluteigriseus* DSM 41896^T (99.24%), *Streptomyces variegatus* NRRL B-16380^T (99.17%) and *Streptomyces flavovariabilis* NRRL B-16367^T (99.17%) comparing with MH257693, MH257695, MH257696, MH257697, and MH257698. Similarities with *Streptomyces kunmingensis* NBRC14463^T (98.82%), *Streptomyces bungoensis* DSM 41781^T(98.76%), *S. chartreusis* NBRC 12753^T (98.69%) and *S. phaeoluteigriseus* DSM 41896^T (98.62%) with MH257694. Whole-genome average nucleotide identity (ANI) values between strain S10^T and *S. chartreusis* NBRC 12753^T, *S. phaeoluteigriseus* DSM 41896^T, *S. variegatus* NRRL B-16380T, *S. flavovariabilis* NRRL B-16367^T, *S. kunmingensis* NBRC 14463^T, *S. bungoensis* DSM 41781^T were 83.63%, 82.89%, 92.55%, 92.51%, 79.29 and 82.87%, respectively, suggesting that the strain S10^T represented a new species. A phylogenetic analysis comparing the S10^T genome with those of 336 other sequenced *Streptomyces* genomes confirmed its relatedness with *Streptomyces variegatus* NRRL B-16380^T and *Streptomyces flavovariabilis* NRRL B-16367^T. Strain S10^T contained LL-diaminopimelic acid in the cell wall. The predominant menaquinones were MK-9(H_6) and MK-9(H_8) and the major fatty acids were iso-$C_{15:0,}$ anteiso-$C_{15:0}$, iso-$C_{16:0}$, and anteiso-$C_{17:0}$. Phospholipids detected were diphosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl choline, three unknown phospholipids, an unknown aminophospholipid and an unknown phosphatidyl glycolipid. On the basis of these genotypic and phenotypic data, it is proposed that isolate S10^T (=JCM 31184^T =CGMCC 4.7315^T) should be classified in the genus *Streptomyces* as *Streptomyces qaidamensis* sp. nov.

INTRODUCTION

The genus *Streptomyces* was first described by Waksman and Henrici.[1] Members of the genus *Streptomyces* are aerobic, Gram-positive filamentous bacteria which can form branched substrate and aerial mycelia. They have LL-diaminopimelic acid with no characteristic sugars in the cell wall[2] and have high genomic DNA G+C contents.[3,4] *Streptomyces* strains are an important source of a broad range of bioactive secondary metabolites, which are widely used in the fields of food, agriculture and pharmaceutical industries.[5,6]

Streptomyces can live in a range of environments, including both fertile and barren soils.[7-9] When we investigated the diversity of actinobacteria in the west of China, a novel actinobacterium producing antibiotic activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated from sand collected from the Qaidam Basin, China. MRSA is a major cause of hospital-acquired infections, and has acquired resistance to many current frontline antibiotic classes, and consequently it is becoming increasingly difficult to combat MRSA infections.[10] The strain S10^T may produce new bioactive compounds with anti-MRSA activity. Consequently, it is of considerable interest for further research.

MATERIALS AND METHODS

Bacterial strains and isolation

Strain S10^T was isolated from a sand sample collected in the Qaidam Basin in Qinghai province, China, by using Gause's synthetic agar medium (20.0 g soluble starch, 1.0 g KNO_3, 0.5 g $K_2HPO_4.3H_2O$, 0.5 g $MgSO_4.7H_2O$, 0.001 g $FeSO_4$, 0.5 g NaCl and 20.0 g agar in 1.0 liter tap water, pH 7.2), supplemented with nalidixic acid (25 μg ml^{-1}) incubated for 7 days at 28 °C. The strain was stored at -86 °C in the presence of 20 % (v/v) glycerol. The reference strains were *Streptomyces chartreusis* NBRC 12753^T, *Streptomyces variegatus* NRRL B-16380^T, *Streptomyces flavovariabilis* NRRL B-16367^T and *Streptomyces kunmingensis* NBRC 14463^T.

Morphological, physiological and biochemical tests

The morphology of spore-chains and hyphae were determined by light microscopy (BH-

2; Olympus) and scanning electron microscopy (SU8010, Hitachi; JSM-5600, JEOL) using cultures grown on Gause's synthetic agar medium at 30 °C for 20 days. Cultural characteristics was examined after growth on standard media ISP 2-7[11], Czapek's agar[12] and nutrient agar after incubation at 30 °C for 14 days. The utilization of sole carbon and nitrogen sources, and metabolism of starch and cellulose, were examined as described previously.[13,14] Growth at various temperatures (4, 10, 15, 20, 30, 37, 40, 45 and 50 °C) and NaCl concentrations (0-10 %) was examined on yeast extract-malt extract (ISP 2). The pH range and the optimum pH were determined by incubating at 28 °C in ISP 2 broth with the pH adjusted to between 4 − 12 by addition of KH_2PO_4/HCl, KH_2PO_4/K_2HPO_4 and K_2HPO_4/NaOH (at intervals of 1.0 pH unit). The antibacterial activity of strain $S10^T$ was determined using a cylinder plug antibacterial bioassay using a clinical methicillin-resistant *Staphylococcus aureus* isolate, a gift of Jodi Lindsay, St George's Hospital, London, as the indicator strain.[15] This indicator strain, EMRSA-8, was resistant to a range of beta-lactam antibiotics, ciprofloxacin, erythromycin, rifampicin, tetracycline and trimethoprim, but sensitive to vancomycin.

Chemotaxonomy

Hyphae for chemotaxonomic studies were prepared by growing the strain in TSB medium in shake flasks for 10 days at 30 °C. The hyphae were harvested by centrifugation and washed twice with distilled water. Then the cells were recentrifuged and freeze-dried. The diaminopimelic acid isomers in the cell wall and whole-cell sugars were analyzed with the method described by Lechevalier and Lechevalier[2] and Staneck and Roberts[16], respectively. The menaquinones were analyzed by the method of Collins *et al.*[17] and analysed by HPLC[18]. The polar lipids were examined using two-dimensional TLC and identified according to method of Minnikin *et al*.[19] The cellular fatty acids methyl esters were extracted by the method of Sasser[20] and analysis by according to the standard protocol of the Sherlock Microbial identification (MIDI) system.[21]

Molecular analysis

The genomic DNA of strain $S10^T$ was extracted and the 16S rRNA gene was amplified

as described by Harunari *et al*.[22] Closely related 16S rRNA gene sequences to that of strain S10^T were identified using the EzTaxon-e server.[23] A phylogenetic tree was generated using the neighbour-joining,[24] maximum-parsimony[25] and maximum-likelihood[26] algorithms in MEGA5.0.[27] Evolutionary distances were calculated using the model of Jukes and Cantor.[28] Topologies of the resultant tree were evaluated by bootstrap analyses[29] based on 1000 resamplings. The G+C content of the DNA was examined by HPLC according to the method of Tamaoka and Komagata.[30] The genomic DNA of strain S10^T was also used to obtain a draft genome sequence using Illumina sequencing; the draft genome consisted of 1 contigs with an estimated genome size of 8.66 Mb(CP015098). The whole-genome average nucleotide identity (ANI) value were calculated by Goris, *et al*. [31] A maximum-composite likelihood tree was performed using PhyloPhlAn and the method of Segata *et al*.[32] Initially, all protein sequences from all annotated *Streptomyces* genomes (.faa files) were retrieved autonomously from the GenBank FTP site (last accessed November 2016) using the term "*Streptomyces*" as a query. Ortholog identification and alignment was performed in Phylophlan using the "-u" command. A maximum likelihood phylogeny was reconstructed from the concatenated alignments in FastTree MP implemented by the Cipres Science Gateway Server[33]. The tree was drawn using the JTT+CAT model with 20 discrete categories (-cat 20). Topology refinement was performed using the follow parameters: -nni 10 -spr 2 -sprlength 10. Nodal support was inferred from 1000 bootstrap pseudoreplicates.

Nucleotide sequence accession number

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S10^T are MH257693- MH257698. The GenBank accession number for the genome of S10^T is CP015098.1.

RESULTS AND DISCUSSION

Morphological, cultural and physiological characteristics

Strain S10^T formed extensively branched substrate hyphae, and the aerial hyphae formed straight spore chains (Figure 1). The spores were cylindrical with a rough-textured surface (Figure 1A). The growth characteristics of strain S10^T cultured on different growth media

were compared to those of the reference strains *Streptomyces chartreusis* NBRC 12753^T, *Streptomyces variegatus* NRRL B-16380^T, *Streptomyces flavovariabilis* NRRL B-16367^T and *Streptomyces kunmingensis* NBRC 14463^T (Table S1). Strain S10^T grew well on ISP medium 2-7, Czapek agar and nutrient agar. Sporulation was poor on ISP 5 and nutrient agar, and no sporulation or growth of aerial hyphae was observed for cultures grown on ISP 6 medium. Black soluble pigments were produced on ISP 6 and brown pigments were produced on nutrient agar. Prior to sporulation, aerial mycelia produced on all media except ISP 6 were white. The morphological features of isolate S10^T were consistent with its classification in the genus *Streptomyces*[34] and were distinct from those of the reference strains.

The physiological properties of strain S10^T, *Streptomyces chartreusis* NBRC 12753^T, *Streptomyces variegatus* NRRL B-16380^T, *Streptomyces flavovariabilis* NRRL B-16367^T and *Streptomyces kunmingensis* NBRC13368^T were compared and found to be different (Table 1). Strain S10^T could utilize L-*myo*-inositol, L-arabinose, D-fructose, D-lactose, D-mannitol, D-raffinose, L-rhamnose or D-xylose as sole carbon sources. It also utilized L-alanine, L-asparagine or L-histidine as sole nitrogen sources, but not L-leucine and L-cysteine (Table 1). The strain S10^T could degrade starch, cellulose, gelatin, tween 20, tween 80 or urea. The temperature range for growth of strain S10^T was 20-40 °C (optimum temperature 30 °C). The pH range for growth was 6–11 (optimum pH 8.0). The maximum NaCl concentration for growth was 10% (w/v) (optimum 1%).

Cylinder plugs of strain S10^T grown for 10 days on ISP 4 medium produced clearing zones of 10 (+/- 2) mm, compared to zones of 13 mm (+/- 2) mm produced when 10 µl of a 50 mg/ml solution of vancomycin was applied to a 10 mm diameter sterile filter disc on the lawn of an MRSA strain bacteria, indicating that the strain S10^T produces antibiotic activity against MRSA.

Chemotaxonomic characteristics

Cell wall analysis indicated that strain S10^T contained LL-diaminopimelic acid, as is characteristic for the genus *Streptomyces*. The whole-cell hydrolysate contained galactose and ribose. The predominant isoprenoid quinone compound were MK-9(H_6) (61.3%) and MK-

9(H_8) (21.5%). The polar lipids detected were diphosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl choline, three unknown phospholipids, an unknown aminophospholipid and an unknown phosphatidyl glycolipid (Figure S1). The major fatty acids were anteiso-$C_{15:0}$ (31.3%), anteiso-$C_{17:0}$ (17.5%), iso-$C_{15:0}$ (12.8%) and iso-$C_{16:0}$ (11.7%); the fatty acid composition was different to *Streptomyces chartreusis* NBRC 12753^T, *Streptomyces variegatus* NRRL B-16380^T and *Streptomyces flavovariabilis* NRRL B-16367^T (Table 2). The DNA G+C content of strain S10^T was 71.3 mol%.

Molecular analysis

There were 6 copies of 16S rDNA in S10^T which were not same exactly. There were high sequence similarities between strain S10^T and *Streptomyces chartreusis* NBRC 12753^T (99.31%), *Streptomyces phaeoluteigriseus* DSM 41896^T (99.24%), *variegatus* NRRL B-16380^T (99.17%) and *Streptomyces flavovariabilis* NRRL B-16367^T (99.17%) comparing with MH257693, MH257695, MH257696, MH257697, and MH257698. Similarities with *Streptomyces kunmingensis* NBRC14463^T (98.82%), *Streptomyces bungoensis* DSM 41781^T(98.76%), *S. chartreusis* NBRC 12753^T (98.69%) and *S. phaeoluteigriseus* DSM 41896^T (98.62%) with MH257694. Based on the 16S rRNA gene sequence, phylogenetic analysis also confirmed that strain S10^T represented a member of the genus *Streptomyces* (Figure 2). The whole-genome average nucleotide identity (ANI) value between strain S10^T and *S. chartreusis* NBRC 12753^T, *S. phaeoluteigriseus* DSM 41896^T, *S. variegatus* NRRL B-16380T, *S. flavovariabilis* NRRL B-16367^T, *S. kunmingensis* NBRC 14463^T, *S. bungoensis* DSM 41781^T were 83.63%, 82.89%, 92.55%, 92.51%, 79.29% and 82.87%, respectively. These values were below the species demarcation threshold of 95–96% ANI suggested for prokaryotic species[35], indicating that S10^T was a new *Streptomyces* species. A phylogenetic tree based on 16S rRNA sequences alone was found to be unstable. As a consequence, a maximum-composite likelihood tree based on analysis of 400 protein sequences from 336 sequenced *Streptomyces* genomes was constructed. This analysis indicated strain S10^T shared highest similarity with *S. variegatus* NRRL B-16380^T and *S. flavovariabilis* NRRL B-16367^T (Figure S2).

Based on its phenotypic, phylogenetic and chemotaxonomic characteristics, strain S10^T represents a novel species within the genus *Streptomyces*, for which the name *Streptomyces qaidamensis* sp. nov. is proposed.

Description of *Streptomyces qaidamensis* sp. nov.

Streptomyces qaidamensis (qai.dam.en'sis. N.L. masc. adj. *qaidamensis* pertaining to Qaidam, China, where the type strain was isolated).

Cells are aerobic and Gram-stain-positive. The substrate hyphae are branched and aerial mycelia formed straight spore chains. The spores are cylindrical with a rough-textured surface. It grow well on ISP medium 2-7, Czapek agar and nutrient agar. Sporulation is poor on ISP 5 and nutrient agar, and not detected on ISP 6. Aerial hyphae are white on all media except ISP 6. Production of black soluble pigments is observed on ISP 6 and brown pigments are produced on nutrient agar. Growth occur between 20-40 °C (optimum temperature 30 °C), at pH 6-11 (optimum pH 8.0) and with 0-10% (w/v) NaCl. It utilize *myo*-inositol, L-arabinose, D-fructose, D-lactose, D-mannitol, D-raffinose, L-rhamnose or D-xylose as sole carbon sources. It also utilize L-alanine, L-asparagine or L-histidine as sole nitrogen sources, but not L-leucine and L-cysteine. The strain S10^T degrade starch, cellulose, gelatin, tween 20, tween 80 or urea. The cell wall contain LL-diaminopimelic acid, galactose and ribose. The predominant isoprenoid quinine compounds are MK-9(H_6) and MK-9(H_8). The polar lipids detected are diphosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl choline, three unknown phospholipids, an unknown aminophospholipid and an unknown phosphatidyl glycolipid. The major fatty acids are anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$ and iso-$C_{16:0}$. The DNA G+C content of strain S10^T is 71.3 mol%.

The type strain, S10^T (=JCM 31184^T =CGMCC 4.7315^T) is isolated from a sand sample collected from the Qaidam Basin, China.

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Table 1. Phenotypic properties of strain S10^T and related type species

Strains: 1, S10^T; 2, *S. chartreusis* NBRC 12753^T; 3, *S. kunmingensis* NBRC13368^T(data from Li *et al*., 2013, Ruan *et al.*, 1985); [36-37] 4, *S. flavovariabilis* B-16367^T (data from Zhang *et al.*, 2016);[38] 5, *S. variegatus* NRRL B-16380^T (data from Zhang *et al.*, 2016).[38] All data were obtained in this study except where indicated otherwise. Abbreviations: +, positive; w, weakly positive; −, negative; N, not determined. All strains were positive for utilization of L-arabinose and D-fructose.

Characteristics	1	2	3	4	5
NaCl for growth(%,w/v)	0-10	0-5	5	>=7	>=7
Carbon source utilization (1.0%, w/v)					
myo-inositol	+	+	+	N	N
D-lactose	+	-	+	-	+
D-raffinose	+	-	+	+	+
L-rhamnose	+	+	+	+	-
D-mannitol	+	+	+	+	-
D-xylose	+	-	+	-	+
Starch	+	+	-	+	+
Nitrogen source utilization (0.1%, w/v)					
L-leucine	-	-	N	N	N
L-cysteine	-	+	N	N	N
L-alanine	+	+	N	N	N
L-asparagine	+	+	N	N	N
L-histidine	+	+	N	N	N
Degradation					
Tween 20	+	-	N	N	N
Tween 80	+	-	N	N	N
Cellulose	+	-	-	-	+
Gelatin liquefaction	+	+	-	+	+
Urease test	+	+	+	+	+

Table 2. Cellular fatty acid composition of strain S10^{T} and related type species.

Strains: 1, S10^{T}; 2, *S. chartreusis* NBRC 12753^{T}; 3, *S. flavovariabilis* B-16367^{T} (data from Zhang *et al.*, 2016);[38] 4, *S. variegatus* NRRL B-16380^{T} (data from Zhang *et al.*, 2016).[38] All data were obtained in this study except where indicated otherwise.

Fatty acid	1	2	3	4
$C_{14:0}$	0.31	0.92	2.7	0.3
$C_{16:0}$	5.89	7.13	8.5	2.9
iso-$C_{14:0}$	1.23	6.36	10.5	2.4
iso-$C_{15:0}$	12.82	5.49	8.9	4.0
anteiso-$C_{15:0}$	31.30	12.09	8.7	27.8
iso-$C_{16:0}$	11.71	32.07	28.8	22.6
iso-$C_{16:1}$ H	1.45	9.59	4.4	5.8
iso-$C_{17:0}$	4.10	0.96	1.1	1.0
cyclo-$C_{17:0}$	ND	1.05	ND	ND
anteiso-$C_{17:0}$	14.53	5.44	1.9	15.5
anteiso-$C_{17:1}$ ω9c	5.09	4.80	1.1	9.0
$C_{17:1}$ ω8c	1.30	0.34	0.2	ND
Sum In Feature 3	4.98	6.58	18.4	3.2
Sum In Feature 9	3.21	2.69	1.5	2.2

ND no-deteced.

The amount of fatty acid was omitted when they all less than 1%.

Summed Feature 3: $C_{16:1}$ ω7c/ω6c or $C_{16:1}$ ω6c/ω7c; Summed Feature 9: $C_{16:0}$ 10-methyl or iso-$C_{17:1}$ω9c.

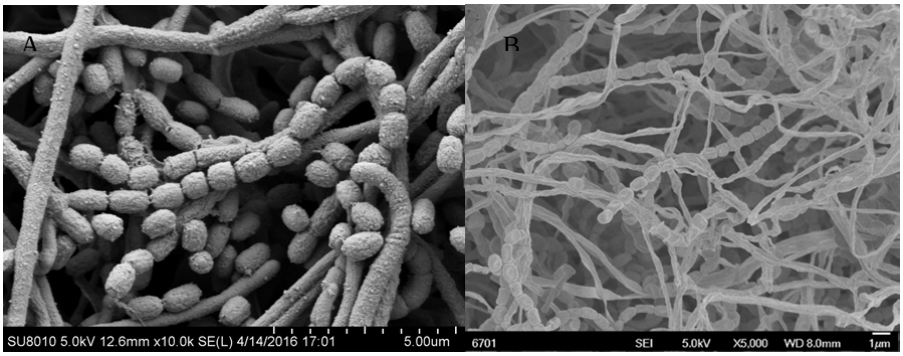

Figure 1 Scanning electron micrograph of strain S10^{T} cultivated on Gause's synthetic agar at 30°C for 20 days. A taking with SU8010, B taking with JSM-5600.

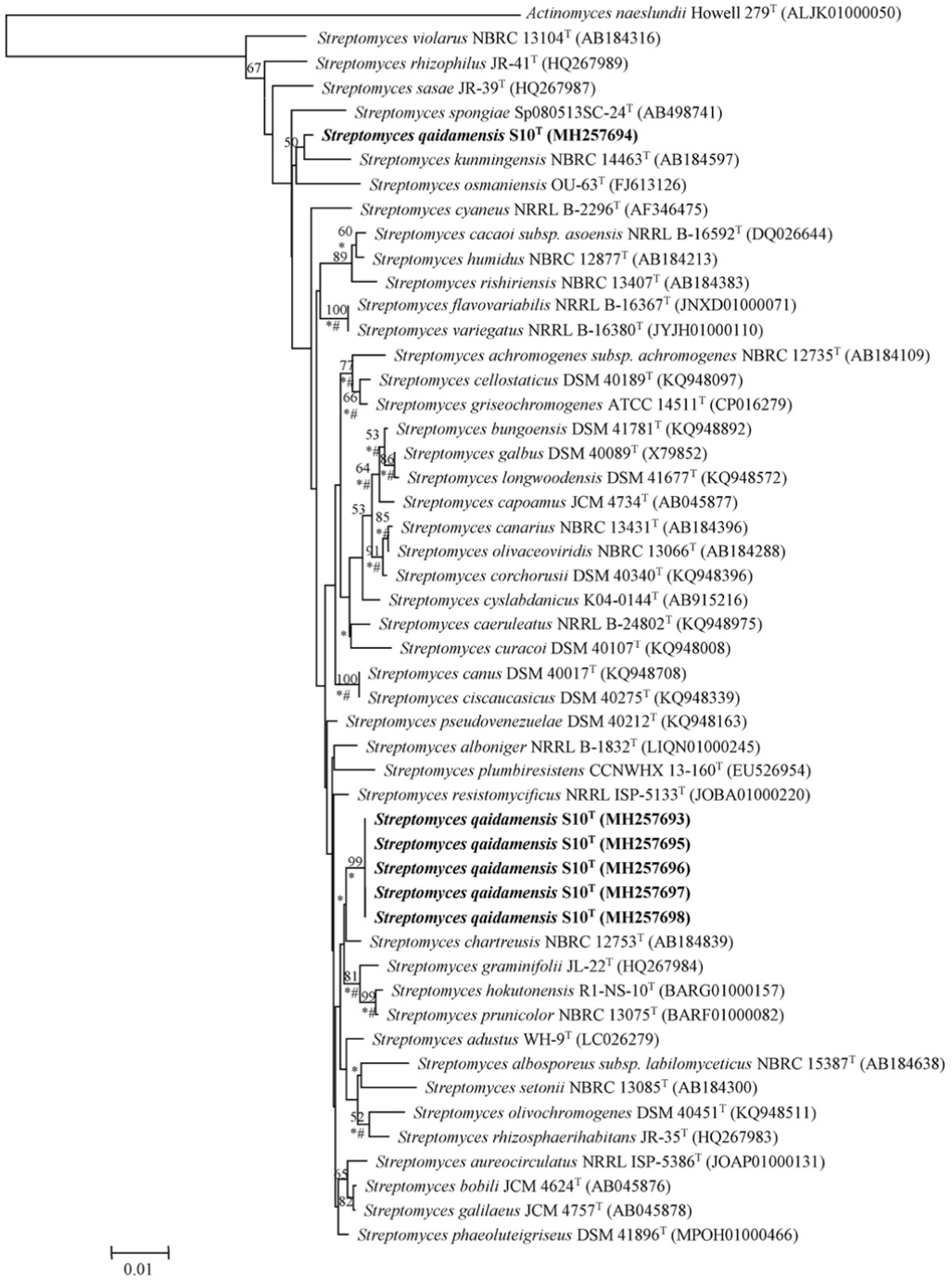

Figure 2 Neighbor-joining phylogenetic tree, based on nearly complete 16S rRNA gene sequences, showing the relationships between strain S10^{T}and strains of related species of the genus *Streptomyces*. Numbers at nodes are bootstrap values based on 1000 re-samplings (only values above 50% are shown).Asterisks(* ,#) indicate that the clades are recovered in maximum-likelihood and maximum-parsimony trees, respectively.