Streptomyces dangxiongensis sp. nov., isolated from soil of Qinghai-Tibet Plateau

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The GenBank/EMBL/DDBJ accession number for the genome and 16S rRNA gene sequence of strain Z022^{T} are SAMN10237529 and KF729589, respectively.

Abstract

A novel actinobacterial strain, designated Z022^{T}, isolated from a soil sample collected from Dangxiong in Tibet Autonomous Region (China), was determined by polyphasic taxonomic approach. The organism had chemotaxonomic and morphological properties consistent with its classification in the genus *Streptomyces.* Strain Z022^{T} showed high similarity value to *Streptomyces lucensis* NBRC 13056^{T} (98.87 %) and *S. achromogenes* subsp. *achromogenes* NBRC 12735^{T} (98.68 %) based on the 16S rRNA gene phylogenetic tree. The genomic DNA G+C content of strain Z022^{T} based on the genome sequence was 72.16 mol%. DNA–DNA relatedness values between strain Z022^{T} and strain *Streptomyces lucensis* NBRC 13056^{T} was 23.7±1.3 % and significantly lower than 70 %. Chemotaxonomic data revealed that strain Z022^{T} possessed MK-9(H_8) and MK-9(H_6) as the predominant menaquinone, LL-diaminopimelic acid as the diagnostic diamino acid, and galactose, glucose, xylose and ribose as whole cell sugars. Diphosphatidylglycerol (DPG) and phosphatidylethanolamine (PE) were the predominant polar lipids; anteiso-$C_{15:0}$, iso-$C_{16:0}$, and anteiso-$C_{17:0}$ were the major fatty acids. On the basis of these genotypic and phenotypic data, it is proposed that isolate Z022^{T} (=JCM 31053^{T} =CGMCC 4.7273^{T}) should be classified in the genus *Streptomyces* as *Streptomyces dangxiongensis* sp. nov.

The genus *Streptomyces* first described by Waksman and Henrici [1]. Members of the genus *Streptomyces* have LL-diaminopimelic acid with no characteristic sugars in the cell wall (wall chemotype I) [2] and have high genomic DNA G+C contents [3, 4]. Species of the genus *Streptomyces* typically have a wide range of metabolic pathways and produce many bioactive secondary metabolites, especially the majority of antibiotics used in medicine [5, 6]. The genus comprises more than 700 species with validly published names at the time of writing the manuscript (www.bacterio.net). There are many *Streptomyces* resource in the minimal human influence area of Qinghai-Tibet Plateau [7-9]. When we investigated the diversity of actinobacteria in the west of China, a novel actinobacteria was isolated from grassland soil collected from the Dangxiong aera on the Qinghai-Tibet Plateau, China.

Strain Z022^{T} was isolated from grass soil sample collected in the Dangxiong, Lhasa city, Tibet Autonomous Region, China on March, 2014. Dangxiong is located in the center of Tibet Autonomous Region. The geographic coordinates of sampling site is 30.33N 91.52 E and the elevation is 4488 m. Strain Z022^{T} was isolated by 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 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 lucensis* JCM 4490^{T} and *Streptomyces achromogenes* subsp. *achromogenes* JCM 4121^{T}. They all came from the Japan Collection of Microorganisms.

Morphological observation of spores and mycelia were conducted by light microscopy (BH-2; Olympus) and scanning electron microscopy (JSM-5600LV; JEOL) using cultures grown on Gause's synthetic agar medium for 20 days. Cultural characteristics was examined by using standard media ISP 2-7 [10], Czapek's agar [11] and nutrient agar after incubation at 30°C for 14 days. Colours were determined according to colour chips from the ISCC-NBS Colour Charts standard samples no.2106 [12]. The utilization of sole carbon and nitrogen, decomposition of starch, cellulose, were examined as described previously [13, 14]. Growth at various temperatures (4, 10, 20, 30, 37, 40, 45 and 50) and NaCl concentrations (0-10 %) were

examined on yeast extract-malt extract (ISP 2). The pH range and the optimum pH were determined by incubating at 30 °C in ISP 2 broth, of which pH was adjusted to 4-12 by addition of KH_2PO4/HCl, KH_2PO_4/K_2HPO_4 and K_2HPO_4/NaOH (at intervals of 1.0 pH unit).

The morphological features of isolate Z022^T were consistent with its classification in the genus *Streptomyces* [15]. The cultural characteristics of strain Z022^T on different kinds of media were presented in Table 1 and microscopic morphology were showed in Fig. 1. Strain Z022^T was an aerobic, Gram-strain-positive and non-motile actinobacterium, which formed branch substrate hyphae and aerial mycelium that differentiates into straight chains with spiny and/or hairy spores. It grew well on ISP medium 2-7, Czapek's agar and nutrient agar. Sporulation was poor on ISP5-6 and Czapek's agar, and can't form on nutrient agar. Aerial mycelia were white on ISP2, ISP4, ISP5, ISP7 and Czapek's agar, grey on ISP3 and light yellow on ISP6 and nutrient agar. Brown diffusible pigments were observed on ISP3. The temperature range for growth of strain Z022^T was 20-40 °C (optimum, 30 °C). The pH range for growth was 5–9 (optimum, pH 7-8). The maximum NaCl concentration for growth was 7 % (w/v) (optimum, 0-2 %). Strain Z022^T could well utilize L-arabinose, D-fructose, D-glucose, D-galactose, weak utilize D-lactose, D-mannitol and D-xylose as sole carbon sources, but not myo-inositol, D-raffinose, L-rhamnose or sucrose. It also utilized L-alanine, L-asparagine, L-histidine and L-cysteine as sole nitrogen sources, but not leucine (Table 2). The strain Z022^T could degrade starch, cellulose, gelatin, tween 20, tween 80 and urea [16-18].

Biomass for chemical studies was prepared by growing the strain in TSB medium in flasks on a rotary shaker at 200 r.p.m for 10 days at 30 °C. Biomass was harvested by centrifugation, washed twice in distilled water, recentrifuged and freezedried. Analysis of the diaminopimelic acid isomers in the cell wall and whole-cell sugars were performed as described previously [2, 19, 20], respectively. The menaquinones were extracted and purified using the method of Collins *et al.* [21] and analysed by HPLC [22]. Polar lipids were extracted, separated by two-dimensional TLC and identified according to procedures outlined by Minnikin *et al.* [23]. Cellular fatty acids were extracted, methylated and separated according to the standard protocol

of the Sherlock Microbial identification (MIDI) system [24, 25] and the fatty acid methyl ester peaks were quantified using the TSBA 5.0 database.

The chemotaxonomic features of strain Z022^T were consistent with the genus *Streptomyces*. The cell wall contained LL-diaminopimelic acid as the diagnostic diamino acid. The galactose, glucose, xylose and ribose present in whole-organism hydrolysates. The predominant isoprenoid quinone compound was MK-9(H_8) (76.3 %) and MK-9(H_6) (23.7 %). The polar lipids were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), two unidentified phospholipids (UPL1-2), two unidentified aminolipids (UAL1-2), and one unidentified phosphoglycolipid (UPGL) (Fig. S1). The major fatty acid found were anteiso-$C_{15:0}$ (25.7 %), iso-$C_{16:0}$ (14.3 %), anteiso-$C_{17:0}$ (11.9 %), anteiso-$C_{17:1}$ *ω9c* (8.4 %), iso-$C_{15:0}$ (7.3 %), iso-$C_{16:1}$ H (6.5 %) and anteiso-$C_{16:1}$ *ω9c* (5.0 %).

The genomic DNA of strain Z022^T was extracted and the 16S rRNA was amplified as described by Mingma *et al.* [26]. Phylogenetic trees were generated using the neighbour-joining [27], maximum-likelihood [28] and maximum-parsimony [29] algorithms in MEGA 5.0 [30]. Evolutionary distances were calculated using the model of Jukes and Cantor [31]. Topologies of the resultant tree were evaluated by bootstrap analyses [32] based on 1000 resamplings. The almost full-length 16S rRNA gene sequence of strain Z022^T (1491 nt) was compared with the corresponding sequences available on the EzTaxon-e server [33]. The result showed that it closely related to *Streptomyces lucensis* NBRC 13056^T (98.88 %), *Streptomyces achromogenes* subsp. *achromogenes* NBRC 12735^T (98.70 %), *Streptomyces cellostaticus* NBRC 12849^T (98.70 %) and *Streptomyces griseochromogenes* NBRC 13413^T (98.70 %). Strains Z022^T and *Streptomyces lucensis* NBRC 13056^T formed an independent clade by using three treeing methods (Fig. 2).

The genome of strain Z022^T was sequenced with Illumina MiSeq platform and PacBio RS II platform. The reads were *de novo* assembled using Newbler (version 2.8) and Hierarchical Genome Assembly Process (HGAP) version 3.0 [34]. The quality of microbial genomes was examined using MUMmer version 3.0 and Pilon software [35, 36]. DNA-DNA hybridization

was performed by Ezaki *et al.* [37]. The average nucleotide identity (ANI) was calculated using the ChunLab's online Average Nucleotide Identity (ANI) calculator [38].

The whole genome of strain Z022^T possessed a chromosome DNA with a size of 8,085,191 bp and a plasmid with a size of 72,694 bp (Table S1). The DNA-DNA relatedness value of strain Z022^T with *Streptomyces lucensis* JCM 4490^T and *Streptomyces achromogenes* subsp. *achromogenes* JCM 4121^T were 23.7±1.3 % and 16.1±1.6 %, respectively, and both values were significantly lower than 70 %, the level considered to be the threshold value for the delineation of genomic species [39]. The whole-genome average nucleotide identity (ANI) value between strain Z022^T and *Streptomyces achromogenes* subsp. *achromogenes* NBRC 12735^T, *Streptomyces cellostaticus* NBRC 12849^T, *Streptomyces griseochromogenes* NBRC 13413^T, *Streptomyces canus* DSM 40017^T, *Streptomyces corchorusii* DSM 40340^T were 87.07 %, 86.02 %, 85.67 %, 82.11 % and 87.87 %, respectively. The DNA G+C content of strain Z022^T was 72.16 mol%.

Generally, there are many differences between the strain Z022^T and the reference strains. For example: the clone colour (Table S1), temperature/pH/NaCl ranges for growth, ability for utilized different sole carbon and nitrogen (Table 1), the relative abundance of major fatty acid (Table 2), the length of genome DNA sequence , genome DNA G+C content (Table S2). Based on the phenotypic, phylogenetic, and chemotaxonomic evidence, strain Z022^T is clearly different from all other species of the genus *Streptomyces*, which supports its classification as a novel species within the genus *Streptomyces*, for which the name *Streptomyces dangxiongensis* sp. nov. is proposed.

Description of *Streptomyces dangxiongensis* sp. nov.

Streptomyces dangxiongensis (dang.xiong.en’sis. N.L. masc. adj. *dangxiongensis* pertaining to Dangxiong, Tibet, China, where the type strain was isolated).

Aerobic, Gram-strain-positive, non-motile, actinobacterium, which forms branch substrate hyphae and aerial mycelium that differentiates into straight chains with spiny and/or hairy spores. Grows well on ISP medium 2-7, Czapek’s agar and nutrient agar. Sporulation is poor on ISP5-6 and

Czapek's agar, and can't form on nutrient agar. Aerial mycelia are whitish on ISP2, ISP4, ISP5, ISP7 and Czapek's agar, grey on ISP3 and light yellow on ISP6 and nutrient agar. Brown diffusible pigments are formed on ISP3. Grows at 20-40°C (optimum, 30 °C), at pH 5-9 (optimum, pH 7-8) and with 0-7 % (w/v) NaCl (optimum, 0-2 %). Well utilizes L-arabinose, D-fructose, D-glucose, D-galactose, weak utilize D-lactose, D-mannitol and D-xylose as sole carbon sources, but not myo-inositol, D-raffinose, L-rhamnose or sucrose. Utilizes L-alanine, L-asparagine, L-histidine and L-cysteine as sole nitrogen sources, but not leucine. The strain Z022^{T} degrades starch, cellulose, gelatin, tween 20, tween 80 and urea. The cell wall contain LL-diaminopimelic acid. The whole-cell sugar pattern mainly consists of galactose, glucose, xylose and ribose. The predominant menaquinones are MK-9(H_8) and MK-9(H_6). The polar lipid profile contained diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), two unidentified phospholipids (UPL1-2), two unidentified aminolipids (UAL1-2), and one unidentified phosphatidylglycolipid (UPGL). The major cellular fatty acids are anteiso-$C_{15:0}$, iso-$C_{16:0}$, and anteiso-$C_{17:0}$.

The type strain, Z022^{T} (=JCM 31053^{T} =CGMCC 4.7273^{T}) was isolated from a soil sample collected from Dangxiong, Tibet, China. The DNA G + C content of the type strain is 72.16 mol%.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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Table 1. Phenotypic properties of strain Z022^T and closely related type species

Strains: 1, Z022^T; 2, *S. lucensis* JCM 4490^T; 3, *S. achromogenes* subsp. *achromogenes* JCM 4121^T; 4, *S. bungoensis* NBRC 15711^T (data from Eguchi *et al.* 1993) [17]; 5, *S. canus* NRRL B-1989 (data from Yan *et al.* 2013) [18]; 6, *S. chartreusis* NBRC 12753^T (data from Lee *et al.* 2014 and Zhang *et al.* 2018) [9, 16]. The data of strain 1, 2 and 3 were obtain in this study. Abbreviations: +, positive; w, weakly positive; − , negative; ND, no data available.

Characteristics	1	2	3	4	5	6
Spore chain	straight	spiral	spiral	spiral	spiral	spiral
Temperature for growth	20-40	10-45	20-40	ND	≤45	≤45
pH range for growth	5-9	6-8	5-10	ND	>5,<9	4.0-12.0
NaCl for growth(%,w/v)	0-7	0-3	0-5	<10,>=7	=3;=4	0-5
Carbon source utilization (1.0%, w/v)						
myo-Inositol	-	w	-	-	-	+
L-Arabinose	+	+	+	+	-	+
D-Fructose	+	w	+	+	+	+
D-glucose	+	+	-	+	-	+
D-Lactose	w	-	-	ND	-	+
D-Galactose	+	+	+	+	-	+
D-Mannitol	w	-	+	+	ND	+
D-Raffinose	-	w	+	-	+	+
L-Rhamnose	-	-	+	-	-	+
Sucrose	-	+	-	w	-	+
D-Xylose	w	+	+	+	-	+
Nitrogen source utilization (0.1%, w/v)						
Leucine	-	w	-	ND	-	+
L-Alanine	+	+	+	ND	-	+
L-Asparagine	+	-	+	ND	-	+
L-Histidine	+	+	+	ND	-	+
L-Cysteine	+	-	-	ND	-	+
Degradation						
Starch	+	+	+	-	ND	+
Cellulose	+	+	+	ND	-	+
Gelatin	+	+	+	+	-	+
Tween 20	+	+	+	ND	-	-
Tween 80	+	+	+	ND	ND	+
Urease test	+	+	+	-	-	+

Table 2. Cellular fatty acid composition of strain Z022^T and related type species.

Strains: 1, Z022^{T}; 2, *S. lucensis* JCM 4490^{T}; 3, *S. achromogenes* subsp. *achromogenes* JCM 4121^{T}; 4, *S. chartreusis* NBRC 12753^{T}. Data for strain 1, 2 and 3 were obtained in this study, for strain 4 was taken from Zhang *et al.* [9]. TR, Trace amount (<1%); -, not detect. Fatty acids amounting to <1% of the total fatty acids in all strains were not shown.

Fatty acid	1	2	3	4
iso-$C_{14:0}$	2.1	TR	1.1	6.4
iso-$C_{15:0}$	7.3	16.0	9.0	5.5
anteiso-$C_{15:0}$	25.7	16.5	30.8	12.1
$C_{16:0}$	4.7	6.7	4.8	7.1
iso-$C_{16:0}$	14.3	11.8	11.1	32.1
iso-$C_{16:1}$ H	6.5	1.9	1.9	9.6
anteiso-$C_{16:1}$ ω9c	5.0	-	-	-
iso-$C_{17:0}$	2.7	7.1	3.7	TR
anteiso-$C_{17:0}$	11.9	15.1	19.5	5.4
cyclo-$C_{17:0}$	TR	TR	TR	1.1
anteiso-$C_{17:1}$ ω9c	8.4	4.9	6.3	4.8
iso-$C_{18:0}$	-	TR	1.5	TR
Sum In Feature 3	-	4.6	1.5	6.6
Sum In Feature 8	2.5	1.6	TR	TR
Sum In Feature 9	4.2	6.0	3.2	2.7

Summed Feature 3: $C_{16:1}$ ω7c/ω6c or $C_{16:1}$ ω6c/ω7c; Summed Feature 8: $C_{18:1}$ ω7c or $C_{18:1}$ ω6c; Summed Feature 9: $C_{16:0}$ 10-methyl or iso-$C_{17:1}$ω9c.

Figure 1 Scanning electron micrograph of strain Z022^{T} cultivated on Gause's synthetic agar at 30°C for 4 weeks.

Figure 2 Neighbor-joining phylogenetic tree, based on nearly complete 16S rRNA gene sequences, showing the relationships between strain Z022^{T} and related species of the genus *Streptomyces*. *Actinomyces naeslundii* Howell 279^{T} was used as an out group. Numbers at nodes are bootstrap values based on 1000 resamplings (only values above 50% are shown).

Figure

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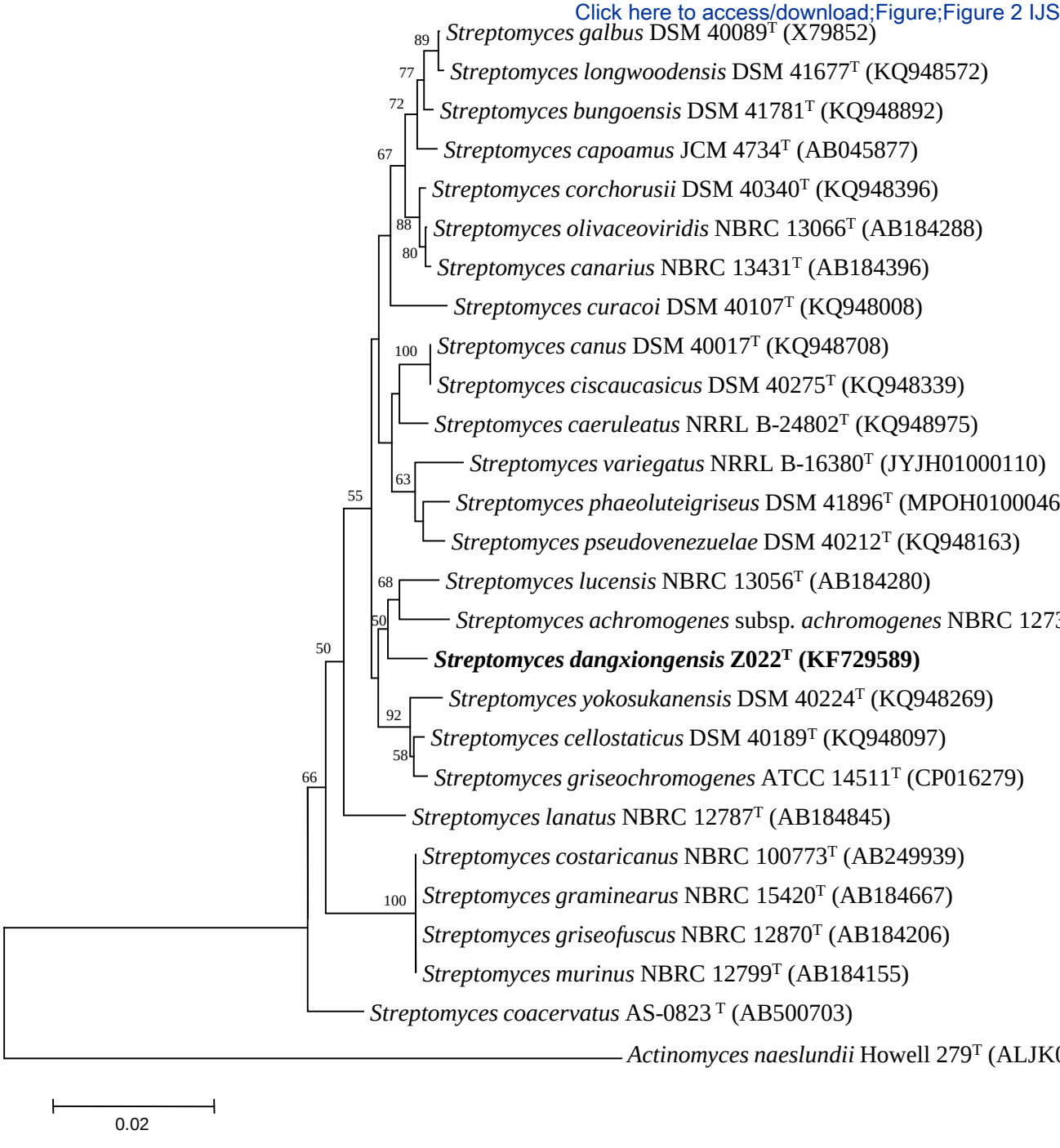

Streptomyces dangxiongensis sp. nov., isolated from soil of Qinghai-Tibet Plateau

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Category: New Taxa (Actinobacteria)

A footnote:

The GenBank/EMBL/DDBJ accession number for the genome and 16S rRNA gene sequence of strain Z022^T are SAMN10237529 and KF729589, respectively.

Supplementary

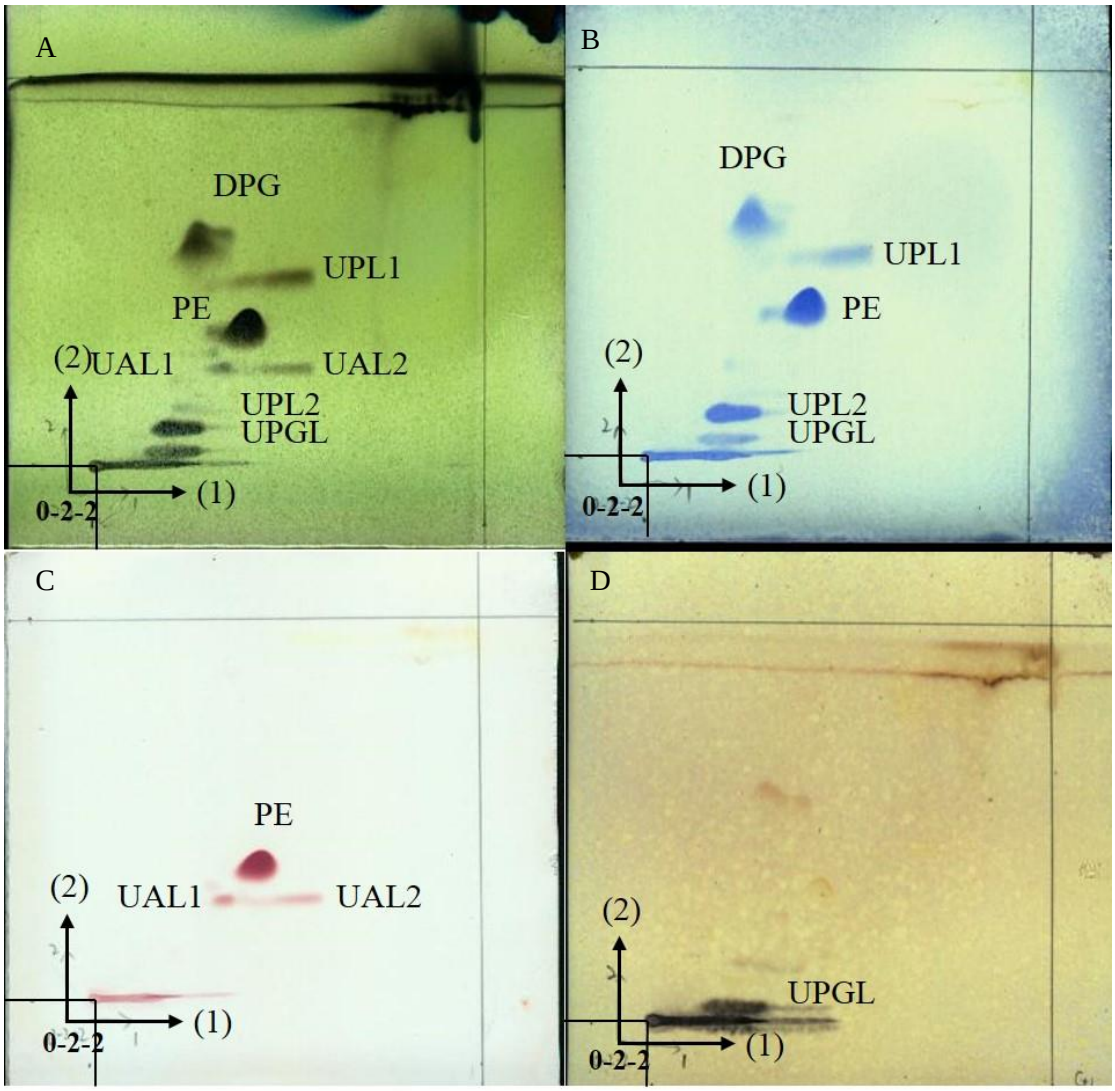

Fig. S1 Polar lipid profiles of strain Z022^T separated by two-dimensional thin layer chromatography and detected with molybdatophosphoric acid (A), molybdenum blue (B), ninhydrin (C) and alpha-naphthol (D). The solvent systems used were as following: Direction 1 was chloroform/methyl alcohol/H_2O (65/25/4, by vol.), Direction 2 was chloroform/ acetic acid/methyl alcohol/H_2O (80/15/12/4, by vol.). Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; UPL(1-2), unidentified phospholipid 1-2; UAL(1-2), unidentified aminolipid 1-2; UPGL, unidentified phosphoglycolipid.

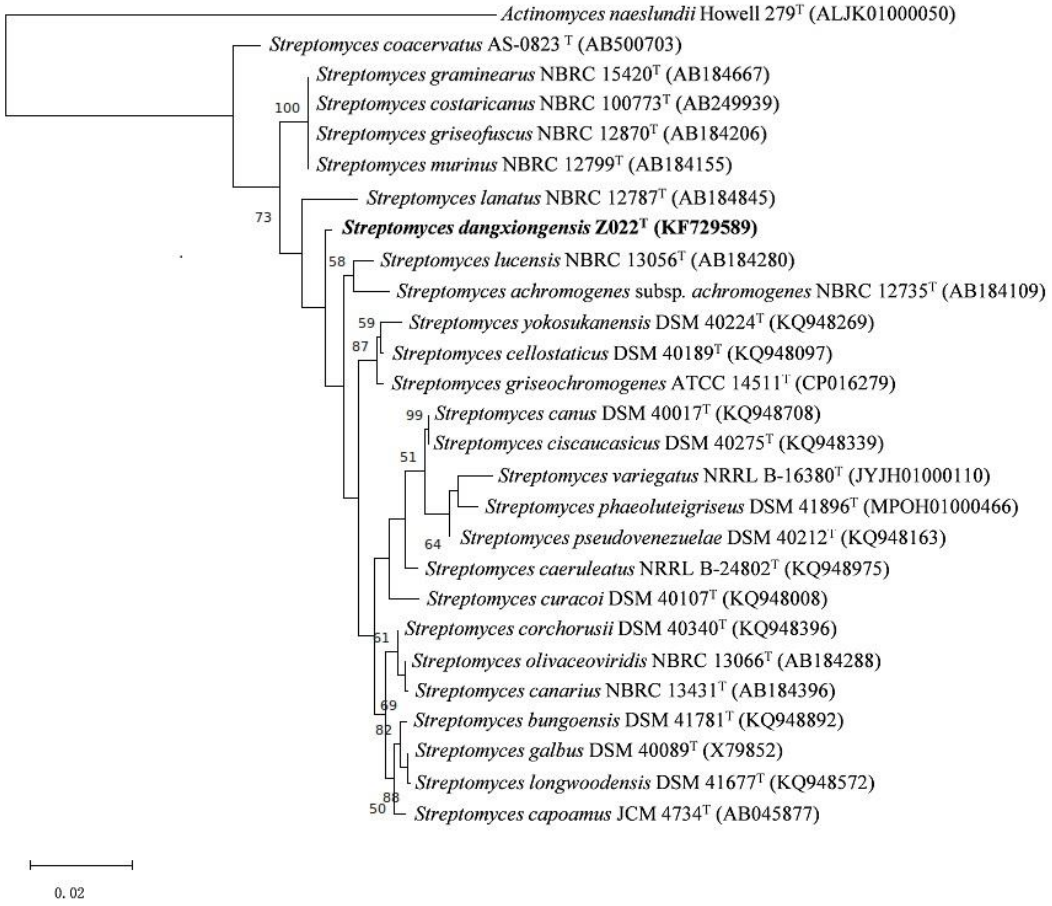

Fig. S2 Maximum-likelihood phylogenetic tree, based on nearly complete 16S rRNA gene sequences, showing the relationships between strain Z022^T and related species of the genus *Streptomyces*. *Actinomyces naeslundii* Howell 279^T was used as an out group. Numbers at nodes are bootstrap values based on 1000 resamplings (only values above 50% are shown).

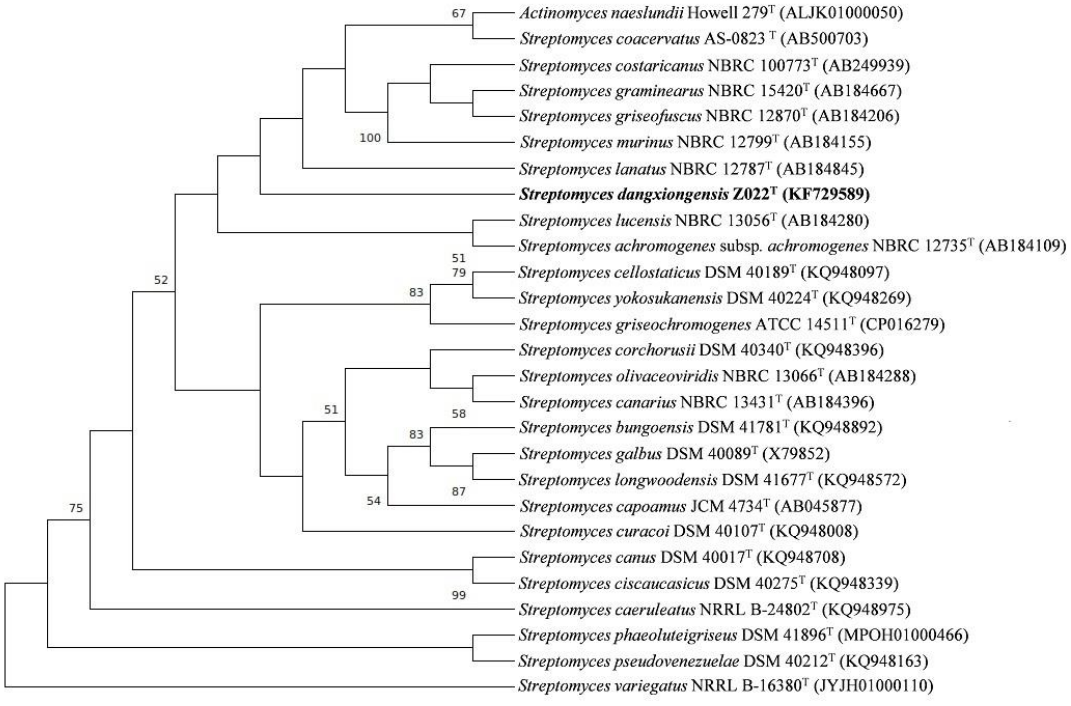

Fig. S3 Maximum-parsimony phylogenetic tree, based on nearly complete 16S rRNA gene sequences, showing the relationships between strain Z022^T and related species of the genus *Streptomyces*. *Actinomyces naeslundii* Howell 279^T was used as an out group. Numbers at nodes are bootstrap values based on 1000 resamplings (only values above 50% are shown).

Table S1. Cultural characteristics of strain Z022^T and closely related type strains on various media at 30°C.

Agar medium	Z022^T	*S. lucensis* JCM 4490^T	*S. achromogenes* subsp. *achromogenes* JCM 4121^T
Yeast extract/extract (ISP2)			
Growth	Good	Good	Good
Sporulation	Good	Poor	Good
Aerial mycelium	White	White	White
Substrate mycelium	Brown	Light yellow	Light brown
Diffusible pigment	None	None	None
Oatmeal (ISP3)			
Growth	Good	Moderate	Good
Sporulation	Good	Moderate	Good
Aerial mycelium	Grey	White	White
Substrate mycelium	Pale grey	Yellow	Light yellow
Diffusible pigment	Brown	None	None
Inorganic salts/starch (ISP4)			
Growth	Good	Good	Good
Sporulation	Good	Moderate	Good
Aerial mycelium	White	Light yellow	White
Substrate mycelium	Light brown	Light yellow	Light yellow
Diffusible pigment	None	None	Black
Glycerol/asparagine (ISP5)			
Growth	Good	Moderate	Moderate
Sporulation	Poor	Moderate	Poor
Aerial mycelium	White	White	White
Substrate mycelium	White	White	White
Diffusible pigment	None	None	None
Peptone/yeast extract/iron (ISP6)			
Growth	Good	Moderate	Moderate
Sporulation	Poor	Poor	Good
Aerial mycelium	Light yellow	White	White
Substrate mycelium	Light yellow	Yellow	Yellow
Diffusible pigment	None	Brown	None
Tyrosine (ISP7)			
Growth	Good	Moderate	Good
Sporulation	Moderate	Moderate	Good
Aerial mycelium	White	White	White
Substrate mycelium	White	White	Light brown
Diffusible pigment	None	None	None
Czapek's agar			
Growth	Good	Moderate	Good
Sporulation	Poor	None	Poor
Aerial mycelium	White	Light Yellow	White
Substrate mycelium	White	Light Yellow	White
Diffusible pigment	None	None	None
Nutrient agar			
Growth	Good	Good	Moderate
Sporulation	None	Moderate	Poor
Aerial mycelium	Light yellow	White	White
Substrate mycelium	Light yellow	White	Light yellow
Diffusible pigment	None	None	None

Table S2. Genome characteristics of strain Z022^{T} and five similar strains

Strains: 1, Z022^{T}; 2, *S. achromogenes* subsp. *achromogenes* NBRC 12735^{T}; 3, *S. cellostaticus* NBRC 12849^{T}; 4, *S. griseochromogenes* NBRC 13413^{T}; 5, *S. canus* DSM 40017^{T}; 6, *S. corchorusii* DSM 40340^{T}. * It is between strain (current line) and Z022^{T}.

Strain	G+C content (mol%)	Genome size (bp)	DDBJ/EMBL/GenBank accession number	ANI*
1	72.16	8,085,191	SAMN10237529	100
2	72.54	8,112,410	GCA_000720835.1	87.07
3	70.98	9,835,283	GCA_001513965.1	86.02
4	70.76	10,764,674	GCA_001542625.2	85.67
5	70.24	11,570,753	GCA_001514145.1	82.11
6	72.03	10,309,547	GCA_001514055.1	87.87