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

Plateau

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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.

1 Abstract

2 A novel actinobacterial strain, designated Z022^T, isolated from a soil sample collected 3 from Dangxiong in Tibet Autonomous Region (China), was determined by polyphasic 4 taxonomic approach. The organism had chemotaxonomic and morphological properties 5 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. 6 7 achromogenes NBRC 12735^T (98.68 %) based on the 16S rRNA gene phylogenetic tree. The 8 genomic DNA G+C content of strain Z022^T based on the genome sequence was 72.16 mol%. 9 DNA-DNA relatedness values between strain Z022^T and strain Streptomyces lucensis NBRC 10 13056^T was 23.7±1.3 % and significantly lower than 70 %. Chemotaxonomic data revealed that 11 strain Z022^T possessed MK-9(H₈) and MK-9(H₆) as the predominant menaquinone, LL-12 diaminopimelic acid as the diagnostic diamino acid, and galactose, glucose, xylose and ribose 13 as whole cell sugars. Diphosphatidylglycerol (DPG) and phosphatidylethanolamine (PE) were 14 the predominant polar lipids; anteiso- $C_{15:0}$, iso- $C_{16:0}$, and anteiso- $C_{17:0}$ were the major fatty acids. 15 On the basis of these genotypic and phenotypic data, it is proposed that isolate Z022^T (=JCM 16 $31053^{T} = CGMCC \ 4.7273^{T}$) should be classified in the genus *Streptomyces* as *Streptomyces* 17 dangxiongensis sp. nov.

18

19 The genus Streptomyces first described by Waksman and Henrici [1]. Members of the genus 20 Streptomyces have LL-diaminopimelic acid with no characteristic sugars in the cell wall (wall 21 chemotype I) [2] and have high genomic DNA G+C contents [3, 4]. Species of the genus Streptomyces 22 typically have a wide range of metabolic pathways and produce many bioactive secondary 23 metabolites, especially the majority of antibiotics used in medicine [5, 6]. The genus comprises 24 more than 700 species with validly published names at the time of writing the manuscript 25 (www.bacterio.net). There are many Streptomyces resource in the minimal human influence 26 area of Qinghai-Tibet Plateau [7-9]. When we investigated the diversity of actinobacteria in 27 the west of China, a novel actinobacteria was isolated from grassland soil collected from the 28 Dangxiong aera on the Qinghai-Tibet Plateau, China.

29 Strain Z022^T was isolated from grass soil sample collected in the Dangxiong, Lhasa city, 30 Tibet Autonomous Region, China on March, 2014. Dangxiong is located in the center of Tibet 31 Autonomous Region. The geographic coordinates of sampling site is 30.33N 91.52 E and the 32 elevation is 4488 m. Strain Z022^T was isolated by Gause's synthetic agar medium (20.0 g 33 soluble starch, 1.0 g KNO₃, 0.5 g K₂HPO₄.3H₂O, 0.5 g MgSO₄.7H₂O, 0.001 g FeSO₄, 0.5 g 34 NaCl and 20.0 agar in 1.0 liter tap water, pH 7.2), supplemented with nalidixic acid (25 µg ml⁻ 35 ¹) incubated for 7 days at 28°C. The strain was stored at -86 °C in the presence of 20 % (v/v) 36 glycerol. The reference strains were *Streptomyces lucensis* JCM 4490^T and *Streptomyces* 37 achromogenes subsp. achromogenes JCM 4121^T. They all came from the Japan Collection of 38 Microorganisms.

39 Morphological observation of spores and mycelia were conducted by light microscopy 40 (BH-2; Olympus) and scanning electron microscopy (JSM-5600LV; JEOL) using cultures 41 grown on Gause's synthetic agar medium for 20 days. Cultural characteristics was examined 42 by using standard media ISP 2-7 [10], Czapek's agar [11] and nutrient agar after incubation at 43 30°C for 14 days. Colours were determined according to colour chips from the ISCC-NBS 44 Colour Charts standard samples no.2106 [12]. The utilization of sole carbon and nitrogen, 45 decomposition of starch, cellulose, were examined as described previously [13, 14]. Growth at 46 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 KH₂PO4/HCl, KH₂PO₄/K₂HPO₄ and K₂HPO₄/NaOH (at intervals of 1.0 pH unit).

50 The morphological features of isolate Z022^T were consistent with its classification in the 51 genus *Streptomyces* [15]. The cultural characteristics of strain Z022^T on different kinds of media 52 were presented in Table 1 and microscopic morphology were showed in Fig. 1. Strain Z022^T 53 was an aerobic, Gram-strain-positive and non-motile actinobacterium, which formed branch 54 substrate hyphae and aerial mycelium that differentiates into straight chains with spiny and/or 55 hairy spores. It grew well on ISP medium 2-7, Czapek's agar and nutrient agar. Sporulation 56 was poor on ISP5-6 and Czapek's agar, and can't form on nutrient agar. Aerial mycelia were 57 white on ISP2, ISP4, ISP5, ISP7 and Czapek's agar, grey on ISP3 and light yellow on ISP6 and 58 nutrient agar. Brown diffusible pigments were observed on ISP3. The temperature range for 59 growth of strain Z022^T was 20-40 °C (optimum, 30 °C). The pH range for growth was 5–9 60 (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 61 62 utilize D-lactose, D-mannitol and D-xylose as sole carbon sources, but not myo-inositol, D-63 raffinose, L-rhamnose or sucrose. It also utilized L-alanine, L-asparagine, L-histidine and L-64 cysteine as sole nitrogen sources, but not leucine (Table 2). The strain Z022^T could degrade 65 starch, cellulose, gelatin, tween 20, tween 80 and urea [16-18].

66 Biomass for chemical studies was prepared by growing the strain in TSB medium in flasks 67 on a rotary shaker at 200 r.p.m for 10 days at 30 °C. Biomass was harvested by centrifugation, 68 washed twice in distilled water, recentrifuged and freezedried. Analysis of the diaminopimelic 69 acid isomers in the cell wall and whole-cell sugars were performed as described previously [2, 70 19, 20], respectively. The menaquinones were extracted and purified using the method of 71 Collins et al. [21] and analysed by HPLC [22]. Polar lipids were extracted, separated by two-72 dimensional TLC and identified according to procedures outlined by Minnikin et al. [23]. 73 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.

76 The chemotaxonomic features of strain Z022^T were consistent with the genus 77 Streptomyces. The cell wall contained LL-diaminopimelic acid as the diagnostic diamino acid. 78 The galactose, glucose, xylose and ribose present in whole-organism hydrolysates. The 79 predominant isoprenoid quinone compound was MK-9(H₈) (76.3 %) and MK-9(H₆) (23.7 %). 80 The polar lipids were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), two 81 unidentified phospholipids (UPL1-2), two unidentified aminolipids (UAL1-2), and one 82 unidentified phosphoglycolipid (UPGL) (Fig. S1). The major fatty acid found were anteiso-83 C_{15:0} (25.7 %), iso-C_{16:0} (14.3 %), anteiso-C_{17:0} (11.9 %), anteiso-C_{17:1} $\omega 9c$ (8.4 %), iso-C_{15:0} 84 (7.3 %), iso-C_{16:1} H (6.5 %) and anteiso-C_{16:1} $\omega 9c$ (5.0 %).

85 The genomic DNA of strain Z022^T was extracted and the 16S rRNA was amplified as 86 described by Mingma et al. [26]. Phylogenetic trees were generated using the neighbour-joining 87 [27], maximum-likelihood [28] and maximum-parsimony [29] algorithms in MEGA 5.0 [30]. 88 Evolutionary distances were calculated using the model of Jukes and Cantor [31]. Topologies 89 of the resultant tree were evaluated by bootstrap analyses [32] based on 1000 resamplings. The 90 almost full-length 16S rRNA gene sequence of strain Z022^T (1491 nt) was compared with the 91 corresponding sequences available on the EzTaxon-e server [33]. The result showed that it 92 closely related to *Streptomyces lucensis* NBRC 13056^T (98.88 %), *Streptomyces achromogenes* subsp. achromogenes NBRC 12735^T (98.70 %), Streptomyces cellostaticus NBRC 12849^T 93 94 (98.70 %) and Streptomyces griseochromogenes NBRC 13413^T (98.70 %). Strains Z022^T and 95 Streptomyces lucensis NBRC 13056^T formed an independent clade by using three treeing 96 methods (Fig. 2).

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

101 was performed by Ezaki et al. [37]. The average nucleotide identity (ANI) was calculated using 102 the ChunLab's online Average Nucleotide Identity (ANI) calculator [38].

103 The whole genome of strain Z022^T possessed a chromosome DNA with a size of 8,085,191 104 bp and a plasmid with a size of 72,694 bp (Table S1). The DNA-DNA relatedness value of 105 strain Z022^T with *Streptomyces lucensis* JCM 4490^T and *Streptomyces achromogenes* subsp. 106 achromogenes JCM 4121^T were 23.7±1.3 % and 16.1±1.6 %, respectively, and both values were 107 significantly lower than 70%, the level considered to be the threshold value for the delineation 108 of genomic species [39]. The whole-genome average nucleotide identity (ANI) value between 109 strain Z022^T and Streptomyces achromogenes subsp. achromogenes NBRC 12735^T, 110 Streptomyces cellostaticus NBRC 12849^T, Streptomyces griseochromogenes NBRC 13413^T, 111 Streptomyces canus DSM 40017^T, Streptomyces corchorusii DSM 40340^T were 87.07 %, 112 86.02 %, 85.67 %, 82.11 % and 87.87 %, respectively. The DNA G+C content of strain $Z022^{T}$ 113 was 72.16 mol%.

114 Generally, there are many differences between the strain Z022^T and the reference strains. 115 For example: the clone colour (Table S1), temperature/pH/NaCl ranges for growth, ability for 116 utilized different sole carbon and nitrogen (Table 1), the relative abundance of major fatty acid 117 (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 118 different from all other species of the genus Streptomyces, which supports its classification as 119 120 a novel species within the genus Streptomyces, for which the name Streptomyces 121 dangxiongensis sp. nov. is proposed.

Description of *Streptomyces dangxiongensis* sp. nov. 122

123 Streptomyces dangxiongensis (dang.xiong.en'sis. N.L. masc. adj. dangxiongensis pertaining to 124 Dangxiong, Tibet, China, where the type strain was isolated).

125 Aerobic, Gram-strain-positive, non-motile, actinobacterium, which forms branch substrate 126 hyphae and aerial mycelium that differentiates into straight chains with spiny and/or hairy spores. 127 Grows well on ISP medium 2-7, Czapek's agar and nutrient agar. Sporulation is poor on ISP5-6 and 128 Czapek's agar, and can't form on nutrient agar. Aerial mycelia are whitish on ISP2, ISP4, ISP5, 129 ISP7 and Czapek's agar, grey on ISP3 and light yellow on ISP6 and nutrient agar. Brown diffusible 130 pigments are formed on ISP3. Grows at 20-40°C (optimum, 30 °C), at pH 5-9 (optimum, pH 7-8) 131 and with 0-7 % (w/v) NaCl (optimum, 0-2 %). Well utilizes L-arabinose, D-fructose, D-glucose, 132 D-galactose, weak utilize D-lactose, D-mannitol and D-xylose as sole carbon sources, but not 133 myo-inositol, D-raffinose, L-rhamnose or sucrose. Utilizes L-alanine, L-asparagine, L-histidine and 134 L-cysteine as sole nitrogen sources, but not leucine. The strain Z022^T degrades starch, cellulose, 135 gelatin, tween 20, tween 80 and urea. The cell wall contain LL-diaminopimelic acid. The whole-136 cell sugar pattern mainly consists of galactose, glucose, xylose and ribose. The predominant 137 menaquinones are MK-9(H₈) and MK-9(H_6). The polar lipid profile contained 138 diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), two unidentified phospholipids 139 (UPL1-2), two unidentified aminolipids (UAL1-2), and one unidentified phosphatidylglycolipid 140 (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 141 142 collected from Dangxiong, Tibet, China. The DNA G + C content of the type strain is 72.16 mol%.

143

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149 **Conflicts of interest**

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

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 committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* 1987;37:463-464.
- 242
- 243
- Table 1. Phenotypic properties of strain Z022^T and closely related type species
- 245 Strains: 1, Z022^T; 2, S. lucensis JCM 4490^T; 3, S. achromogenes subsp. achromogenes JCM
- 246 4121^T; 4, *S. bungoensis* NBRC 15711^T (data from Eguchi *et al.* 1993) [17]; 5, *S. canus* NRRL
- 247 B-1989 (data from Yan *et al.* 2013) [18]; 6, *S. chartreusis* NBRC 12753^T (data from Lee *et al.*
- 248 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	250
Spore chain	straight	spiral	spiral	spiral	spiral	spiral
Temperature for growth	20-40	10-45	20-40	ND	≤45	25⊥ ≤45
pH range for growth	5-9	6-8	5-10	ND	>5,<9	4. 2-52 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)						253
myo-Inositol	-	W	-	-	-	זבֿ⊿
L-Arabinose	+	+	+	+	-	234
D-Fructose	+	w	+	+	+	255
D-glucose	+	+	-	+	-	+
D-Lactose	w	-	-	ND	-	256
D-Galactose	+	+	+	+	-	257
D-Mannitol	w	-	+	+	ND	257
D-Raffinose	-	W	+	-	+	258
L-Rhamnose	-	-	+	-	-	+
Sucrose	-	+	-	w	-	259
D-Xylose	w	+	+	+	-	260
Nitrogen source utilization (0.1%, w/v)						200
Leucine	-	W	-	ND	-	261
L-Alanine	+	+	+	ND	-	+
L-Asparagine	+	-	+	ND	-	2 ⁺ 2 ⁺ 22
L-Histidine	+	+	+	ND	-	263
L-Cysteine	+	-	-	ND	-	+
Degradation						264
Starch	+	+	+	-	ND	2 ⁺ 265
Cellulose	+	+	+	ND	-	262
Gelatin	+	+	+	+	-	266
Tween 20	+	+	+	ND	-	-
Tween 80	+	+	+	ND	ND	267
Urease test	+	+	+	-	-	

269

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

- 271 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-C16:1 w9c	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} w9c	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

275 Summed Feature 3: $C_{16:1} \omega 7c/\omega 6c$ or $C_{16:1} \omega 6c/\omega 7c$; Summed Feature 8: $C_{18:1} \omega 7c$ or $C_{18:1} \omega 6c$; 276 Summed Feature 9: $C_{16:0}$ 10-methyl or iso- $C_{17:1}\omega 9c$.

277

278

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

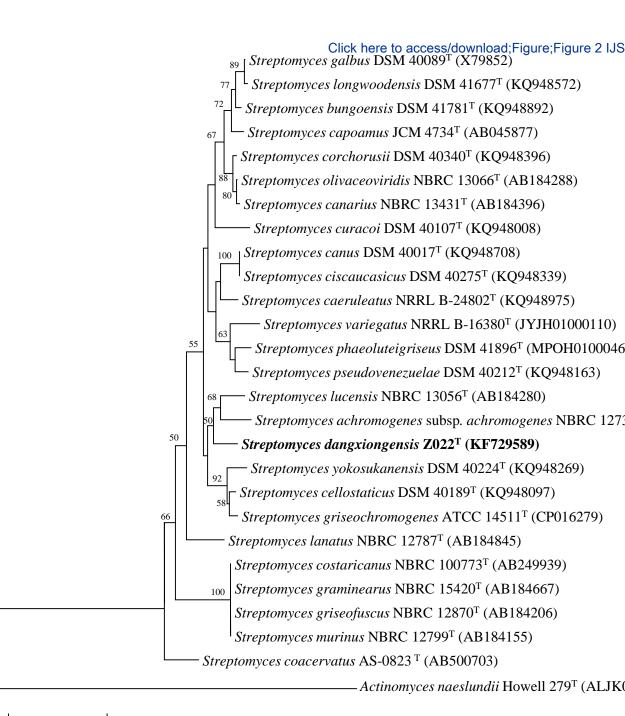
sequences, showing the relationships between strain Z022^T and related species of the genus

283 *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).

285

Figure



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

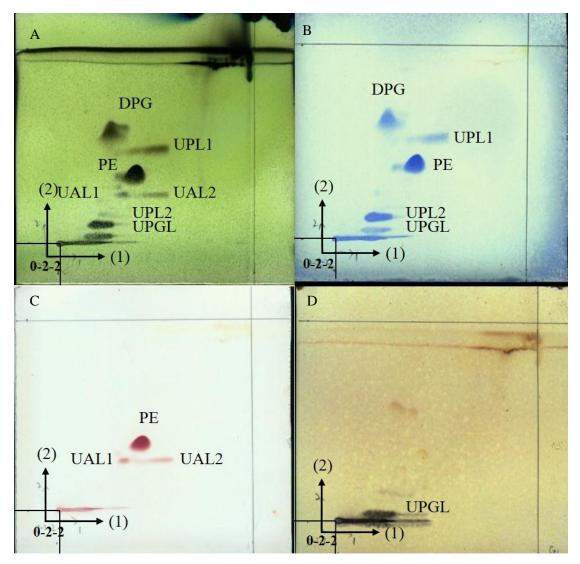


Fig. S1 Polar lipid profiles of strain $Z022^{T}$ separated by two-dimensional thin layer chromatography and detected with molybdatophosphoric acid (A), molybdenum blue (B), ninhydrin (C) and alpha-naphthol (D). The solvent systems used were as following: Direction 1 was chloroform/methyl alcohol/H₂O (65/25/4, by vol.), Direction 2 was chloroform/ acetic acid/methyl alcohol/H₂O (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.

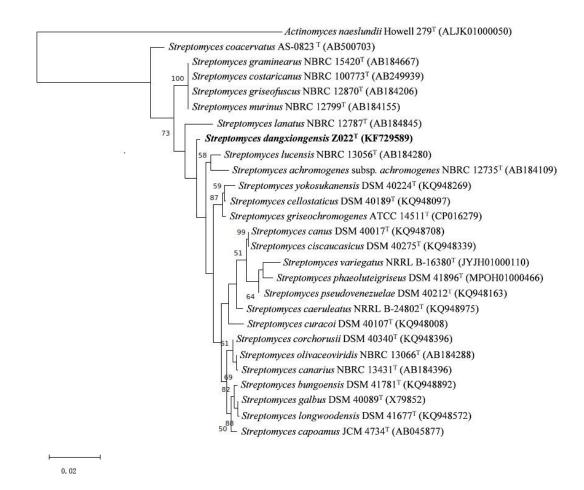


Fig. S2 Maximum-likelihood phylogenetic tree, based on nearly complete 16S rRNA gene sequences, showing the relationships between strain Z022^T and related species of the genus *Streptomyces. Actinomyces naeslundii* Howell 279^T was used as an out group. Numbers at nodes are bootstrap values based on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

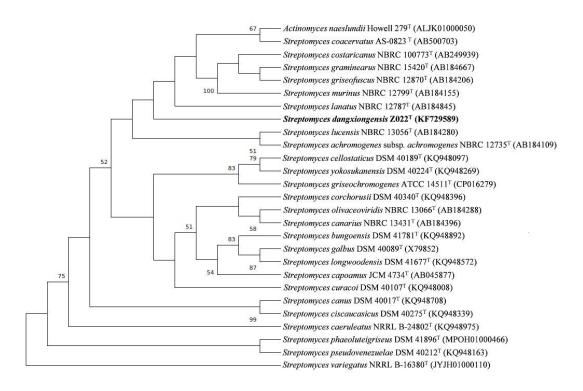


Fig. S3 Maximum-parsimony phylogenetic tree, based on nearly complete 16S rRNA gene sequences, showing the relationships between strain Z022^T and related species of the genus *Streptomyces. Actinomyces naeslundii* Howell 279^T was used as an out group. Numbers at nodes are bootstrap values based on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

Agar medium	Z022 ^T	S. lucensis	S. achromogenes subsp.	
Versteret/seteret/ISD2)		JCM 4490 ^T	achromogenes JCM 4121 ^T	
Yeast extract/extract (ISP2)	Carl	Caral	Card	
Growth Sporulation	Good Good	Good	Good Good	
1	White	Poor White	White	
Aerial mycelium				
Substrate mycelium	Brown None	Light yellow None	Light brown	
Diffusible pigment	INOILE	None	None	
Oatmeal (ISP3) Growth	Carl	Madausta	Card	
Sporulation	Good Good	Moderate Moderate	Good Good	
1		White	White	
Aerial mycelium	Grey			
Substrate mycelium	Pale grey	Yellow	Light yellow	
Diffusible pigment	Brown	None	None	
Inorganic salts/starch (ISP4)	Cort	Cert	01	
Growth	Good	Good	Good	
Sporulation	Good	Moderate	Good	
Aerial mycelium	White Light brown	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)	a 1			
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 S1. Cultural characteristics of strain $Z022^{T}$ and closely related type strains on various media at 30° C.

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

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

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.