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ORIGINAL PAPER

Streptomyces desertarenae sp. nov., a novel actinobacterium isolated from a desert sample

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Abstract A *Streptomyces* isolate, designated strain SYSU D8023^T, was isolated from a desert sand sample collected from Gurbantunggut desert, China. The characterisation of the isolate was achieved using a polyphasic taxonomic approach. The isolate was found to be Gram-positive and aerobic. The strain was found to be able to grow at 14–50 °C, pH 6.0–9.0 and in the presence of up to 7% (w/v) NaCl. Strain SYSU D8023^T contains LL-diaminopimelic acid as a cell wall diamino acid. The polar lipids were identified as diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, an unidentified glycolipid and an unidentified phospholipid. MK-9(H_6) and MK-9(H_8) were detected as the respiratory quinones, and anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$ as the predominant fatty acids. Pairwise comparison of the 16S rRNA gene sequences indicated that strain SYSU D8023^T has a sequence identity of 97.9% to *Streptomyces barkulensis* RC 1831^T. The DNA G + C content of strain SYSU D8023^T was determined to be 70.1 mol%. Based on the analyses of the phenotypic, genotypic and phylogenetic characteristics, strain SYSU D8023^T was concluded to represent a novel species of the genus *Streptomyces*, for which the name *Streptomyces desertarenae* sp. nov. is proposed.

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The type strain of the species is SYSU D8023^T (= CGMCC 4.7455^T = KCTC 49023^T).

Keywords *Streptomyces desertarenae* sp. nov. · Gurbantunggut desert · Polyphasic taxonomy

Introduction

Members of the genus *Streptomyces* has been extensively studied for their capacity to produce numerous natural products (Nimaichand et al. 2013; Tamreihao et al. 2016; Nithya et al. 2018). While the genus is ubiquitous in nature, its study has been mostly limited to soil (Goodfellow and Fiedler 2010; Katz and Baltz 2016). Unlike other environments, deserts provide a unique ecosystem in that they are characterised by an arid environment, with water and nutrients being their limiting factors (Andrew et al. 2012). A study on microbial diversity in the Atacama Desert has revealed the dominance of *Streptomyces* among other actinobacteria (Okoro et al. 2009). This was further illustrated by the isolation of several novel *Streptomyces* species from the Atacama Desert including *Streptomyces asenjonii*, *Streptomyces atacamensis* and *Streptomyces deserti* (Santhanam et al. 2012a, b; Goodfellow et al. 2017).

The genus *Streptomyces* was first introduced by Waksman and Henrici (1943) and, at the time of writing, there are more than 800 validly named species (http://www.bacterio.net/streptomyces.html). A major characteristic of the members of the genus *Streptomyces* is the presence of LL-diaminopimelic acid in the cell wall peptidoglycan, along with an absence of characteristic cell wall sugars (Lechevalier and Lechevalier 1970).

This paper describes another member of the genus *Streptomyces* isolated from a desert sample.

Materials and methods

Isolation and preservation

Sand samples were collected from Gurbantunggut desert (44°39′N, 87°11′E) located in China. Isolation of strain SYSU D8023^T was based on the method described by Yang et al. (2017). Following purification, pure cultures was maintained on Reasoner's 2A (R2A) agar and preserved as glycerol suspensions (20%, v/v) at − 80 °C. The basal growth condition of the strain for all experiments was maintained at pH 7.0 and 28 °C, unless otherwise stated.

S. barkulensis DSM 42082^T was obtained from DSMZ, Germany and cultured under comparable conditions as a reference strain.

Phenotypic characteristics

For phenotypic characteristics, strain SYSU D8023^T was cultured on R2A agar. Morphological characteristics were observed using a light microscope (BH2; Olympus) and a scanning electron microscope (JSM-6330F, JOEL), following 7 days of incubation. Growth conditions, colours of the mycelia and pigment formation were recorded after 2 weeks growth on International *Streptomyces* Project (ISP) media, Czapek's agar, Gause's agar, R2A agar, nutrient agar and Potato Dextrose Agar. Gram reaction was tested using a Gram Stain Solution Kit (Shanghai Yeasen Biotechnology Co., Ltd.) and confirmed by a non-staining procedure (Buck 1982). Growth temperature range (0, 4, 14, 28, 37, 45, 50, 55, 60 and 65 °C) and NaCl tolerance (media supplemented with NaCl up to 30% w/v concentration) were observed on R2A agar after 2 weeks of incubation. For pH tolerance, R2A broth was prepared between pH 4.0–10.0 (with interval of 1.0 pH unit, using the buffer system described by Nie et al. 2012) and growth observed after 2 weeks of incubation. Oxidase and catalase activities were determined by assessing the oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine (Kovacs 1956) and the formation of bubbles on addition of 3% H_2O_2, respectively. Cellulose, gelatin and starch hydrolysis, H_2S production, milk coagulation and peptonisation, nitrate reduction, degradation of Tweens (20, 40, 60 and 80) and urease activity were tested as previously described (McFaddin 1976; Gonzalez et al. 1978). Utilisation of carbon and nitrogen sources were examined by using the medium of Smibert and Krieg (1994).

Chemotaxonomy

Biomass for chemical studies of strain SYSU D8023^T was obtained from cultures grown in R2A broth for

Table 1 Differential phenotypic characteristics between strain SYSU D8023^T and the closely related type strain *S. barkulensis* DSM 42082^T

Characteristics	1	2
Growth at		
14 °C	+	−
45 °C	+	−
Hydrolysis of		
Starch	−	+
Tween 80	−	+
Nitrate reduction	+	−
Utilisation of		
Lactose	−	+
Cellulose	−	+
Arabinose	+	−
Fructose	−	+
Maltose	−	+
Rhamnose	+	−
Saccharose	−	+
Glucose	−	+
Mannose	−	+
Xylitol	−	+
Threonine	+	−
Lysine	+	−
Phenylalanine	+	−
Polar lipids	DPG, PE, PG, PI, PIM	DPG, PG, PI, PIM
Major fatty acid(s) (> 5%)	anteiso-$C_{15:0}$, iso-$C_{16:0}$; anteiso-$C_{17:0}$, $C_{12:0}$	iso-$C_{16:0}$; anteiso-$C_{15:0}$; anteiso-$C_{17:0}$; iso-$C_{15:0}$;
DNA G + C content (mol%)	70.1	66.2

Strains: 1, SYSU D8023^T; 2, *S. barkulensis* DSM 42082^T. All data, except for those relating to polar lipids and DNA G + C content of the reference strain (Ray et al. 2014), are from this study

+ Positive, − negative, *DPG* diphosphatidylglycerol, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannoside, *GL* unidentified glycolipid and *PL* unidentified phospholipid

7 days, unless otherwise mentioned. Respiratory quinones were extracted from lyophilised cells (Collins et al. 1977), purified and analysed by HPLC (Tamaoka et al. 1983). Polar lipids were extracted, separated and examined by two-dimensional TLC procedure on silica gel G_{60} plates (Merck) (Minnikin et al. 1979). Cells for cellular fatty acid analysis were obtained by culturing strain SYSU D8023^T and *S. barkulensis* DSM 42082^T on tryptic soy agar (TSA, Difco) for 7 days. Cellular fatty acids were extracted, methylated and analysed following the instructions of the Sherlock Microbial Identification System (MIDI) version 6.1 and the TSBA6 database (Sasser 1990). Diaminopimelic acid was analysed according to the procedures developed by Hasegawa et al. (1983). Whole cell sugars were analysed as described by Lechevalier and Lechevalier (1980). The genomic DNA G + C content was determined by HPLC after enzymatic degradation (Mesbah et al. 1989).

Molecular characterisation

Extraction of chromosomal DNA, PCR amplification of 16S rRNA gene and sequencing of the purified products were carried out as described by Li et al. (2007). The phylogenetic relationships of strain SYSU D8023^T were determined after BLAST (Altschul et al. 1990) searches of the 16S rRNA gene sequences in NCBI and the EzBioCloud databases (Yoon et al. 2017). 16S rRNA gene sequences of closely related

Table 2 Complete fatty acid profiles of strains SYSU D8023^T and *Streptomyces barkulensis* DSM 42082^T

Fatty acids	SYSU D8023^T	DSM 42082^T
$C_{10:0}$ 3OH	2.8	1.1
iso-$C_{12:0}$	1.8	0.8
$C_{12:0}$	**6.2**	2.9
$C_{11:0}$ 2OH	1.3	0.6
iso-$C_{13:0}$	–	0.2
anteiso-$C_{13:0}$	–	0.4
iso-$C_{14:0}$	2.5	4.3
$C_{14:0}$	–	0.3
iso-$C_{15:0}$	3.9	**6.5**
anteiso-$C_{15:0}$	**20.3**	**20.2**
$C_{14:0}$ 2OH	1.2	0.6
iso-$C_{16:1}$ H	2.7	4.7
iso-$C_{16:0}$	**17.8**	**27.3**
anteiso-$C_{16:0}$	–	0.5
$C_{16:0}$	4.2	3.3
anteiso-$C_{17:1}$ ω9c	3.9	1.2
iso-$C_{17:0}$	5.3	3.6
anteiso-$C_{17:0}$	**16.2**	**9.4**
$C_{17:1}$ ω8c	0.7	0.3
iso -$C_{18:0}$ H	–	0.3
$C_{18:3}$ ω6,9,12c	–	1.0
$C_{18:0}$	0.7	1.2
$C_{18:1}$ 2OH	4.7	2.4
iso-$C_{20:0}$	0.8	0.5
Summed feature 3[a]	0.9	2.8
Summed feature 8[a]	–	2.6
Summed feature 9[a]	3.0	1.4

Numbers in bold characters indicate fatty acids with composition higher than 5% of the total fatty acids. Values below 0.1 are excluded from the list

[a]Summed features represent two or three fatty acids that cannot be separated by the MIDI System. Summed feature 3: $C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH; Summed feature 8: $C_{18:1}$ ω7c and/or $C_{18:1}$ ω6c; Summed feature 9: methyl-$C_{16:0}$ 10 and/or iso-$C_{17:1}$ ω9c

type strains were retrieved for multiple alignments (CLUSTAL X software package, Thompson et al. 1997) and generation of phylogenetic dendrograms (MEGA version 7.0, Kumar et al. 2016). Algorithms based on the neighbour-joining (Saitou and Nei 1987), maximum-parsimony (Fitch 1971) and maximum-likelihood (Felsenstein 1981) methods were used for generation of phylogenetic trees. Evolutionary distances in the neighbour-joining and maximum likelihood trees were calculated using Kimura two-parameter model (Kimura 1980, 1984). The topology of each tree was evaluated by bootstrap analysis of 1000 replications (Felsenstein 1985).

Results and discussion

Phenotypic characteristics

Strain SYSU D8023^T was observed to grew well on ISP 3, ISP 4, ISP 6, ISP 7, Czapeks' agar, nutrient agar and Gause's agar, moderately on ISP 5 and poorly on ISP 2 and Potato Dextrose agar. The colour of the aerial mycelia on the above media were white while the substrate mycelia on ISP 3, ISP 4, ISP 7 and NA showed a gray colour. No soluble pigments were produced on the media tested. The aerial mycelia were observed to bear rectiflexibles spore chains (see supplementary Fig. S1). Strain SYSU D8023^T was observed to grow at 14–50 °C, pH 6.0–9.0 and in the presence of up to 7% NaCl, Optimum growth was observed at 28 °C, pH 7.0 and in the absence of NaCl. Phenotypic properties distinguishing strain SYSU D8023^T from the closely related strain *S. barkulensis* DSM 42082^T are listed in Table 1. Other detailed phenotypic characteristics of strain SYSU D8023^T are provided in the species description below.

Chemotaxonomic characteristics

The respiratory quinones of strain SYSU D8023^T were found to be MK-9(H_6) (83.0%) and MK-9(H_8) (17.0%). The polar lipid profile of strain SYSU D8023^T was found to contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, an unidentified phospholipid and an unidentified glycolipid (Fig. S2). Strain SYSU D8023^T was found to contain LL-diaminopimelic acid as cell wall diamino acid. The whole cell sugars were identified as glucose and fucose. The major cellular fatty acids (> 5%) detected were anteiso-$C_{15:0}$ (20.3%), iso-$C_{16:0}$ (17.8%), anteiso-$C_{17:0}$ (16.2%) and $C_{12:0}$ (6.2%). Detailed fatty acid profiles of the strain SYSU D8023^T and the closely related type strain *S. barkulensis* DSM 42082^T are given in Table 2. The genomic

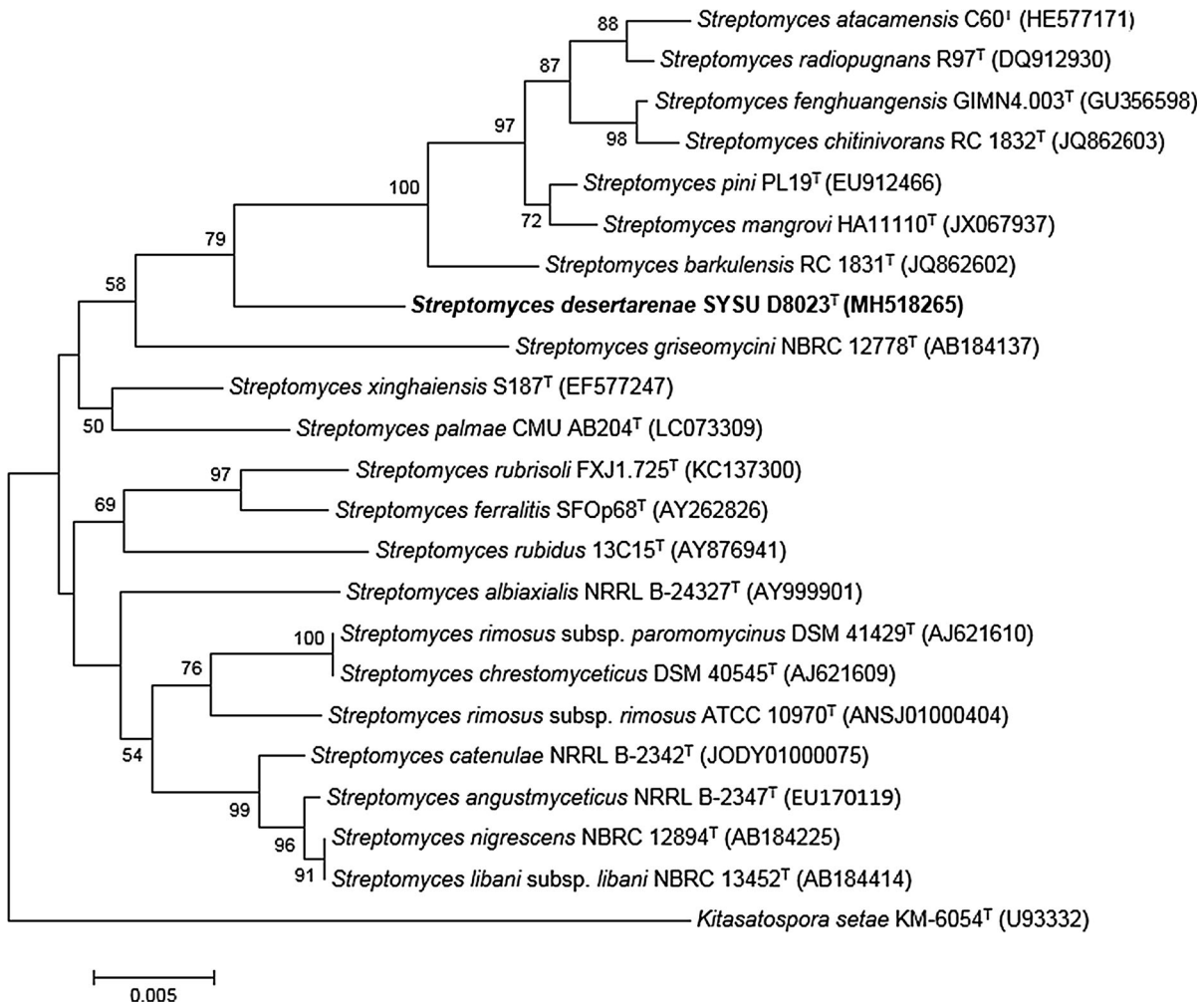

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships of strain SYSU D8023^T among related members of the genus *Streptomyces*. Bootstrap values ($\geq$ 50%) based on 1000 resamplings are given at the nodes. Bar, 0.005 substitutions per nucleotide position

DNA G + C content of strain SYSU D8010^T was determined to be 70.1 mol%.

Molecular characteristics

On pairwise comparison with the 16S rRNA gene sequences available in the EzBioCloud database, strain SYSU D8023^T showed highest 16S rRNA gene sequence identity with *S. barkulensis* RC 1831^T (97.9%). This relationship was further supported by the phylogenetic trees (Figs. 1, S3, S4). Since the 16S rRNA gene sequence identities of strain SYSU D8023^T with all current validly named *Streptomyces* strains were below 98.65%, DNA-DNA hybridization experiments were not performed with the related strains (Chun et al. 2018).

On the basis of 16S rRNA gene sequence analysis and chemotaxonomic features, strain SYSU D8023^T can be considered to be a member of the genus *Streptomyces*. The strain can, however, be differentiated from the closely related type strain *S. barkulensis* DSM 42082^T (= RC 1831^T) by several characteristics as listed in Table 1. For example, strain SYSU D8023^T can grow at 14 °C and 45 °C, but could not tolerate NaCl above 8%, unlike the reference strain *S. barkulensis* DSM 42082^T. Furthermore, strain SYSU

D8023^T contained phosphatidylethanolamine as one of its polar lipid, unlike *S. barkulensis* DSM 42082^T. Based on these characteristics and phylogenetic analyses, strain SYSU D8023^T is considered to represent a novel species of the genus *Streptomyces*, for which the name *Streptomyces desertarenae* sp. nov. is proposed. The Digital Protologue database (Rosselló-Móra et al. 2017) TaxoNumber for strain SYSU D8023^T is TA00589.

Description of *Streptomyces desertarenae* sp. nov.

Streptomyces desertarenae (de.sert.a.re'nae. L. neut. n. *desertum* desert; L. fem. n. *arena* sand; N.L. gen. n. *desertarenae* of desert sand, referring to the source of the type strain).

Aerobic, Gram-positive, halotolerant actinomycete. Forms well-developed and extensively branched substrate mycelia. Aerial mycelia form rectiflexibles spore chains; spores are non-motile, rough and short rod shaped. Shows good growth on ISP 3, ISP 4, ISP 6, ISP 7, Czapek's agar, nutrient agar and Gause's agar, moderate growth on ISP 5 and poor growth on ISP 2 and Potato Dextrose agar. Growth occurs at 14–50 °C (optimum, 28 °C), pH 6–9 (optimum, pH 7) and in the presence of up to 7% (w/v) NaCl. Positive for nitrate reduction but negative for oxidase, catalase and urease activities, milk coagulation and peptonisation, and H_2S production. Hydrolyses Tweens 20 and 40 but not cellulose, gelatin, starch or Tweens 60 and 80. Utilises arabinose, galactose, glycerol, inositol, mannitol, melitriose, rhamnose, sodium pyruvate, sodium succinate, sorbitol and trehalose as sole carbon sources but not cellobiose, fructose, glucose, lactose, maltose, mannose, ribose, saccharose, sodium fumarate, sorbose, xylitol or xylose. Utilises alanine, arginine, aspartic acid, glutamine, hypoxanthine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine as sole nitrogen source but not adenine, cysteine, glycine or histidine. The diagnostic cell wall diamino acid is LL-diaminopimelic acid. Contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, an unidentified phospholipid and an unidentified glycolipid as polar lipids. MK-9(H_6) and MK-9(H_8) are the respiratory quinones. Major cellular fatty acids are $C_{12:0}$, anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$. The DNA G + C content of the type strain is 70.1 mol%.

The type strain, SYSU D8023^T (= KCTC 49023^T = CGMCC 4.7455^T), was isolated from a sample collected from Gurbantunggut desert in China. The GenBank accession number for the 16S rRNA gene sequence of strain SYSU D8023^T is MH518265.

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Authors' contribution NS and WJL conceived the study. LYL, ZWY, MDA and BZF performed research. LYL, ZWY, DHMA and NS analysed data. ZWY, NS and WJL wrote the paper. All authors approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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