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ORIGINAL PAPER

Streptomyces gulbargensis sp. nov., isolated from soil in Karnataka, India

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Abstract During the course of screening for industrially important microorganisms, an alkali-tolerant and thermotolerant actinomycete, strain DAS 131^T, was isolated from a soil sample collected from the Gulbarga region, Karnataka province, India. The strain was characterized by a polyphasic approach that showed that it belonged to the genus *Streptomyces*. Growth was observed over a wide pH range (pH 6–12) and at 45°C. The 16S rRNA gene sequence of strain DAS 131^T was deposited in the GenBank database under the accession number DQ317411. 16S rRNA gene sequence analysis revealed that strain DAS 131^{T}was most closely related to *Streptomyces venezuelae* ISP 5230^T (AY999739) with a sequence similarity of 99.5% (8 nucleotide differences out of 1,477). Despite this very high sequence similarity, strain DAS 131^T was phenetically distinct from *S. venezuelae*. The DNA relatedness between these strains was 54%, indicating that strain DAS 131^T is a distinct genomic species. On the basis of phenetic and genetic analyses, strain DAS 131^T is classified as a new species in the genus *Streptomyces*, for which we propose the name *Streptomyces gulbargensis* sp. nov.

Keywords *Streptomyces gulbargensis* sp. nov · Polyphasic approach · 16S rRNA

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Introduction

Actinomycetes are widely distributed in natural and man-made environments and play an important role in the degradation of organic matter (Lechevalier and Lechevalier 1967; Goodfellow and Williams 1983). They are well known as a rich source of antibiotics and bioactive molecules and they are thus considered to be a rich biotechnological resource (Chun et al. 1997; Labeda et al. 1997; Iwai and Takahashi 1992). The genus *Streptomyces* is the largest antibiotic producing genus in the microbial world. Members of this genus probably constitute the largest actinomycete group in soils (Saadoun and Gharaibeh, 2002). As well as antibiotics, members of the genus *Streptomyces* are renowned for the production an array of industrially important

metabolites (Goodfellow and Simpson 1987), including herbicides, enzymes, enzyme inhibitors and insecticides. *Streptomyces* systematics has been improved by the application of a polyphasic approach, which includes the use of molecular techniques (Korn-Wendisch and Kutzner 1992).

In the course of screening for industrially important actinomycetes, an actinomycete, designated strain DAS 131, was isolated from soil collected from semi-arid soils of Gulbarga, northern Karnataka province India. Strain DAS 131 is highly alkalitolerant and thermotolerant. The strain was characterized by the polyphasic approach. The results indicate that strain DAS 131 belongs to the genus *Streptomyces*. This paper reports a taxonomic analysis of the strain DAS 131. On the basis of phenetic and genetic analyses, strain DAS 131^{T} is classified as a new species in the genus *Streptomyces*, for which we propose the name *Streptomyces gulbargensis* sp.nov.

Material and methods

Bacterial Cell Culture: Strain DAS 131 was isolated from soil collected from the Gulbarga region by a standard serial dilution technique using starch casein agar medium (Kuster and Williams 1964; Nolan and Cross 1988). Cultures were incubated at 28°C for 2 to 3 weeks.

Morphological, physiological and biochemical characterization of strain DAS 131 were studied by following the guidelines of the *International Streptomyces* project (Shirling and Gottelieb 1966, 1968a, b). Modified ISP 2 medium was used for determining the pH tolerance of strain DAS 131.The slide and plate cultures were prepared aseptically by using starch casein and glycerol asparagine agar for micro-morphological studies and photographic records using optical and scanning electronic microscopy (Pridham et al. 1957, 1958).

Macro-morphological, cultural, physiological and biochemical characterizations of DAS 131 were performed according to method proposed by Pridham and Gottlieb (1948) and Shirling and Gottlieb (1966). Color determination was compared with color chips from the ISCC-NBS COLOR CHARTS standard sample No.2106 (Kelly 1964).

Chemotaxonomy

The cell wall fraction was purified and analyzed by the method of Lechevalier and Lechevalier (1980). The procedure of Becker et al. (1965) and Lechevalier and Lechevalier (1980) were used to determine the whole-cell sugar composition. Menaquinones were determined according to the Collins (1985) method. Phospholipids were determined according to Lechevalier and Lechevalier (1980). DNA for the G+C content was obtained by the method of Marmur (1961). Fatty acid composition was analyzed by following the procedure of Komagata and Suzuki (1987) and Takeuchi and Hatano (1998).

16S rRNA gene sequence

The chromosomal DNA of strain DAS 131 was isolated as described by Hopwood et al. (1985). The 16S rRNA gene was amplified by PCR using a PCR kit (Sino-American Biotechnology Co., Beijing), primer A-8-27f (5′-AGAGTTTGATCC TGGCTCAG-3′) and primer B-1523-1504r (5′-AAGGAGGTGATCCAGCCGCA-3′; *Escherichia coli* numbering (Brosius et al. 1978). The conditions used for thermal cycling were as follows: denaturation at 95°C for 5 min, followed by 35 cycles consisting of denaturation at 95°C for 1 min, primer annealing at 56°C for 1 min and primer extension at 72°C for 3 min. At the end of the cycles, the reaction mixture was kept at 72°C for 5 min and then cooled to 4°C. About 1.5 kb amplified 16S rRNA gene fragment was separated by agarose gel electrophoresis. The purified fragment was used for sequencing as described previously (Cui et al. 2001).

Sequence alignment and phylogenetic analysis

The almost complete 16S rRNA gene sequence of strain DAS 131 was aligned manually with sequence of related *Streptomyces* from the GenBank database. The evolutionary tree was inferred using the neighbor joining method (Saitou and Nei

1987). *Streptomyces nodosus* was used as the outgroup, from the evolutionary distance data corrected by Kimura's 2-parameter model (Kimura 1980). The topology of the resultant tree was evaluated by bootstrap analysis (Felsenstein 1985) of the neighbor-joining method based on 1,000 resamplings. The Clustal X program (Thompson et al. 1997) and the software package MEGA version 2.1 (Kumar et al. 2001) was used for multiple alignment and phylogenetic analysis. Tree view programme (Page 1996) was used to display, edit and print the phylogenetic tree.

Nucleotide sequence accession number

The almost complete 16S rRNA sequence of strain DAS 131 determined in this study (1,477 nucleotides) has been deposited in the GenBank database. The Accession No. for strain DAS 131 is DQ 317411.

Results and discussion

Morphological observation of cultures of strain DAS 131 after 7–15 days of growth showed abundant aerial and vegetative hyphae, which were well developed and did not exhibit fragmentation. Smooth, oval spores were borne in long, straight-to-flexuous chains (Fig. 1). Growth of aerial and substrate mycelium was found on all media tested. Morphological characteristics on different media are shown in Table 1. The aerial mycelium of strain DAS 131 varied from white to dark pink on different medium. The substrate mycelium differs from yellow pink to brown yellow. Diffusible pigments were observed (Table 1), and melanin was produces on peptone yeast extract iron agar.

The cell wall of strain DAS 131 contained LL-DAP and glycine, which indicates that it has a cell wall chemotype I (Lechevalier and Lechevalier 1970a, b). No diagnostic sugars were found in whole cell hydrolysates. The diagnostic phospholipids were phosphatidylethanolamine and phosphatidylcholine. The predominant menaquinones were MK-9 (H_4)-11%, MK-9 (H_6)-71% and MK-9 (H_8)-18%. The major fatty acids were anteiso $C_{15:0}$ (26%), iso $C_{16:0}$(20%), iso$C_{15:0}$ (10%), anteiso $C_{17:0}$ (9%) and $C_{16:0}$ (8%).

The phenotypic characteristics showed that strain DAS 131 belongs to the genus *Streptomyces*. The G+C content of DNA of the strain DAS 131 is 69.8 mol%. An almost complete 16S rRNA gene sequence for strain DAS 131 was determined in this study. A phylogenetic tree was constructed based on 16S rRNA gene sequences to show the comparative relationship between strain DAS 131 and other related *Streptopmyces* species (Fig. 2). The comparative analysis of 16S rRNA gene sequence and phylogenetic relationship showed that strain DAS 131 lies in a subclade in the tree with *Streptomyces venezuelae* ISP 5230^T with which it shares a 16S rRNA gene sequence similarity of 99.5% (8 nucleotide differences out of 1,477). A comparative phenetic study between strain DAS131^T and closely related species of the genus was performed to differentiate the strain DAS 131^T from *Streptomyces venezuelae* ISP 5230^T (Table 2). DNA-DNA hybridization between strain DAS 131 and *Streptomyces venezualae* ISP 5230^T was carried out by applying the optical renaturation method (De Ley et al. 1970; Huss et al. 1983; Jahnke 1992) under optimal hybridization conditions. The determined DNA-DNA relatedness value of 54% (54 ± 0.3, duplicate experiments) was significantly lower than 70%, which is considered

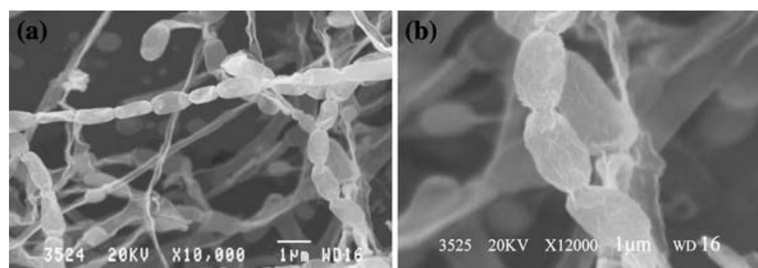

Fig. 1 Scanning electron microscopic view of spore arrangement in strain DAS 131^T

Table 1 Culture characteristics of strain DAS 131

Agar medium	Color of mycelium		Soluble pigment
	Aerial	Substrate	
Yeast Extract Malt Extract (ISP 2)	White	Yellow	Brown
Oat Meal (ISP 3)	Pink	Yellow	Absent
Inorganic Salts Starch (ISP 4)	Brown	Light Yellow	Absent
Glycerol Asparagine (ISP 5)	White	Yellow	Absent
Tyrosine (ISP 7)	Creamy White	Brown	Yellow
Starch Casein	Pink	Yellow	Absent
Czapek's	White	Light Pink	Light Yellow
Glucose Asparagine	Pink	Light Yellow	Brown

ISP media (Shirling and Gottlieb 1966) were used; colors were obtained from Kelly (1964)

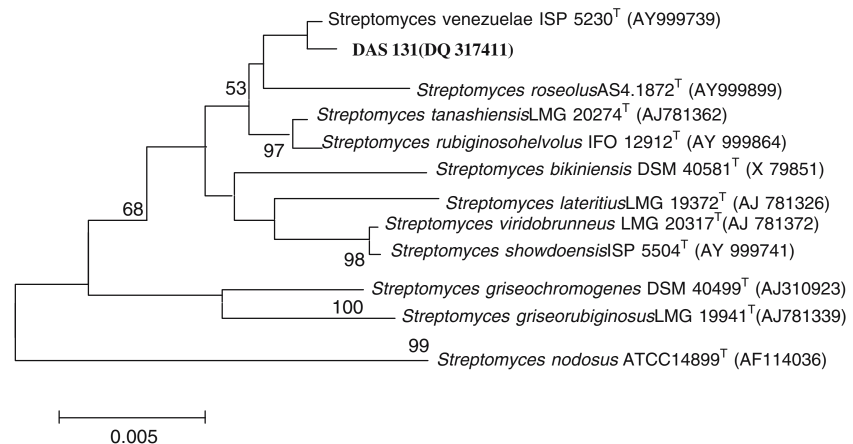

Fig. 2 Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA gene sequence, showing the position of strain DAS 131 among its phylogenetic neighbours. Numbers at the branch nodes are boot strap values, expressed as a percentage of 1,000 replicates (only values above 50% are shown). Bar 0.005 substitution per nucleotide position

to be the threshold value for the delineation of genomic species (Wayne et al. 1987). On the basis of phylogenetic, morphological and chemotaxonomical evidence and its physiological and biochemical distinctiveness (Williams et al. 1983), it is proposed that strain DAS 131 be classified as *Streptomyces gulbargensis sp. nov*.

Description of *Streptomyces gulbargensis sp. nov*.

Streptomyces gulbargensis(gul.barg.en'sis.N.L. masc.adj.gulbargensis pertaining to Gulbarga region, a place of north Karnataka, India).

Gram-positive, aerobic, non-motile, alkalitolerant and thermotolerant with good growth in hyphae without fragmentation. Long, smooth, oval-shaped spores are arranged in straight chains. Grows well on almost all media tested, namely ISP2, ISP3, ISP4, ISP5, ISP7, Starch casein agar, Czapek's agar and glucose asparagine agar, colonies are whitish to pink, smooth, powdery. 0.5–1.0 mm in diameter. Starch, casein and cellulose are degraded. Positive for nitrate reduction and gelatin liquefaction. Temperature range for growth is 28°C–45°C with an optimum at 39°C–42°C. Strain DAS 131 grows at pH 6–12 and in the presence of 7% NaCl. Good growth was found on almost all carbon sources tested including D-glucose, D-galactose, D-Arabinose, D-xylose, D-mannitol, L-rhamnose, raffinose, *meso*-inositol, and sucrose. Acid is not produced from these carbon sources. Strain DAS 131^T contains LL-diaminopimelic acid, no diagnostic sugars in the whole cell hydrolysates. Predominant phospholipids are phosphatidylgylcerol, phosphatidylcholine, phosphatidylethanolamine and phosphatidylmethylethanolamine. The major menaquinones are MK-9 (H_4)-11%, MK-9 (H_6)-71% and MK-9

Table 2 Characteristics that distinguish strain DAS 131^{T} from closely related Streptomyces species

Characteristics	DAS 131	*S.venezualae**	*S.roseulus**	*S.tanasninsis**	*S.lavendulae**
Colony Morphology	Pink	Gray to Brown	Light Pink	Pale Pink	White to Pink
Spore Shape	Smooth	Smooth	Smooth	Smooth/rough	NA
Spore chain morphology	Straight	Straight	Straight (RF)	Flexuous	Spiral
Production of diffusible pigment	Brown	Dark Brown	Pale Yellow	Brown	Absent
Melanoid pigment	+	+	–	+	+
Milk coagulation	+	–	–	–	–
Milk peptonization	–	+ (Slow)	–	ND	+
Starch hydrolysis	+	–	+	+	+
Gelatin Liquefaction	+	+	–	+	+
Casein hydrolysis	+	–	+	–	+
Utilization of					
D-glucose	+	+	+	+	+
D-galactose	+	+	+	+	+
D-arabinose	+	+	+	+	+
D-xylose	+	+	+	+	+
raffinose	+	–	–	+	–
meso-inositol	+	–	–	–	–
D-mannitol	+	–	–	–	–
Sucrose	+	+	–	+	+
L-rhamnose	+	–	+	+	–
Growth at 45°C	+	–	–	ND	ND
Growth at pH					
6	+	+	+	–	+
8	+	+	+	+	+
10	+	+/ –	–	+	+
12	+	–	ND	ND	ND

+: Utilization, – : no utilization, NA: Not available, ND: not determined

Colony morphology was observed on Starch casein agar. Diffusible pigments were observed on ISP 5. Melanin was observed on peptone yeast extract iron agar and tyrosine agar (ISP 7)

*Sources of the data, Shirling and Gottlieb (1968a, b, 1969)

(H_8)-18%. The major fatty acids are iso$C_{15:0}$(10%), anteiso$C_{15:0}$(26%), iso$C_{16:0}$ (20%), anteiso$C_{17:0}$(9%) and $C_{16:0}$(8%). The DNA G+C content is 69.8 mol%. The type strain is DAS 131^{T}. Isolated from soil collected from the Gulbarga region of Karnataka province, southern India. The type strain is DAS 131^{T}(=CCTCC AA-206001^{T} = KCTC-19179^{T}).

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