Streptomyces deccanensis sp. nov., an alkaliphilic species isolated from soil

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A novel actinomycete strain, DAS-139^T, was isolated from a soil sample collected from Gulbarga, Karnataka Province, India. The isolate was characterized by white to grey aerial mycelium. Long spore chains were found on the aerial mycelium and the aerial mycelium was composed of non-motile spores with hairy surfaces. The cell wall of strain DAS-139^T contained LL-diaminopimelic acid isomer as the diagnostic diaminoacid indicating that the cell wall was of chemotype-I. The predominant menaquinones were MK-9(H₆) (76 %), MK-9(H₄) (14 %) and MK-9(H₈) (10 %). Phosphatidylethanolamine was the diagnostic phospholipid. On the basis of 16S rRNA gene sequence, phenotypic and phylogenetic analyses, the novel strain was identified as a member of the genus *Streptomyces*. The novel strain grew optimally at 28 °C and pH 9.0. The G+C content of the genomic DNA was 71.8 mol%. 16S rRNA gene sequence analysis showed that the novel isolate had 99.4 % sequence similarity with *Streptomyces scabiei* ATCC 49173^T and 99.2 % similarity with *Streptomyces diastachromogenes* ATCC 12309^T. Furthermore, DNA–DNA hybridization with these two *Streptomyces* species showed 36.0 and 43.0 % relatedness, respectively. Based on these observations, strain DAS-139^T is proposed to represent a novel species of the genus *Streptomyces*, for which the name *Streptomyces deccanensis* sp. nov. is proposed with the type strain DAS-139^T (=KCTC 19241^T=CCTCC AA 207004^T).

The genus *Streptomyces* represents a group of micro-organisms that is widely distributed in nature. The genus *Streptomyces* remains a focus of systematic research, not only because members of this genus are still the most promising sources of commercially significant compounds, but also because current molecular biological methods are having an increasing impact on conventional *Streptomyces* systematics based on phenotypic characteristics (Williams *et al.*, 1983). A polyphasic approach with a judicious combination of genotypic and phenotypic features can provide useful information for the classification of this complex group and for the recognition of novel species. Molecular systematic methods, notably 16S rRNA gene sequencing, and phylogenetic analysis are having an increasing impact on the systematics of the genus *Streptomyces* (Kim *et al.*, 1998, 2000; Kim & Goodfellow, 2002; Li *et al.*, 2002). The application of chemical methods

has influenced the development of actinomycete systematics at the genus level and even at higher taxonomic levels (Goodfellow & Cross, 1984; Kroppenstedt, 1985; Minnikin *et al.*, 1980; Stackebrandt, 1986). With more than 500 recognized species and subspecies, the taxon currently harbours the largest number of species with validly published names in the domain *Bacteria* (Hain *et al.*, 1997). The present study describes a distinct alkaliphilic actinomycetes strain, strain DAS-139^T, as a novel species of the genus *Streptomyces* based on a polyphasic approach.

Strain DAS-139^T was isolated from a soil sample taken from the Gulbarga region, Karnataka Province, Deccan Trap, India, after 2 weeks incubation at 28 °C on glycerol-asparagine agar [ISP medium 5 (Shirling & Gottlieb, 1966)]. Morphological, physiological and biochemical characterization of strain DAS-139^T was carried out by following the standard protocol of the International *Streptomyces* Project (Shirling & Gottlieb, 1966, 1968a, b). For colour determination, samples were compared with colour chips from ISCC-NBS Color Charts standard sample no. 2106 (Kelly, 1964). Microscopic observation

Abbreviation: ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DAS-139^T is EF219459.

of spores and mycelia of strain DAS-139^T grown on ISP 2 for 21 days were made by light microscopy (BH-2; Olympus) and scanning electron microscopy (JSM-5600LV; JEOL). The novel strain was examined for a range of phenotypic properties using standard procedures (Shirling & Gottlieb, 1966; Williams *et al.*, 1983), except that pH was regulated by using autoclaved Na₂CO₃. The following buffers were used to control the pH range; pH 6.0, 7.0 and 8.0, 0.1 M KH₂PO₄/0.1 M NaOH; pH 9.0 and 10.0, 0.1 M NaHCO₃/0.1 M Na₂CO₃; pH 11.0, 0.05 M Na₂HPO₄/0.1 M NaOH and pH 12.0, 0.2 M KCl/0.2 M NaOH. Strain DAS-139^T was incubated in liquid ISP 2 medium for 2–3 weeks, pH was regulated after sterilization of the media to pH 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 and 12.0 using autoclaved KOH, K₂CO₃, NaOH or Na₂CO₃. Tolerance to temperature and NaCl was determined on modified ISP 2 medium followed by incubation at 28 °C for 3–4 weeks.

Cell-wall amino acid was purified and analysed by the methods of Jiang *et al.* (2001). The procedure of Lechevalier & Lechevalier (1980) was used for analysis of whole-cell sugars. Menaquinones and phospholipids were extracted from freeze-dried biomass and purified according to Collins (1985) and Minnikin *et al.* (1984) and finally analysed by HPLC. Fatty acid composition was determined by following the procedures of Komagata & Suzuki (1987) and Takeuchi & Hatano (1998).

Genomic DNA was extracted and the 16S rRNA gene was amplified as described by Cui *et al.* (2001). The DNA G+C content of strain DAS-139^T was determined using the thermal denaturation method (Marmur & Doty, 1962). Reference strains were chosen from BLAST (Altschul *et al.*, 1997) search results. Phylogenetic analysis was performed using the software package MEGA version 2.1 (Kumar *et al.*, 2001) after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). A phylogenetic tree was reconstructed using the neighbour-joining method of Saitou & Nei (1987) from *K*_{nuc} values (Kimura, 1980). The topology of the phylogenetic tree was evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

Morphological observation of a 21 day culture of strain DAS-139^T grown on yeast extract/malt extract agar (ISP 2 medium) revealed that strain DAS-139^T had the typical characteristics of the genus *Streptomyces*. Aerial mycelium and substrate mycelium were well-developed without fragmentation. Long chains of spores were straight, hairy and non-motile (Fig. 1). Strain DAS-139^T could grow well at between pH 7.0 and 12.0, with an optimum pH value of 9.0–10.0. The optimum growth temperature was 28 °C. Strain DAS-139^T grew in presence of 0–5 % NaCl (w/v). Mycelium of strain DAS-139^T was well developed on most media tested including yeast extract/malt extract agar (ISP 2), oatmeal agar (ISP 3), glycerol-asparagine agar (ISP 5), tyrosine agar (ISP 7) and Czapek's agar. The novel strain showed poor growth on inorganic salt-starch agar (ISP 4)

Fig. 1. Scanning electron micrographs indicating the spore chain morphology of strain DAS-139^T. (a) Spores in straight chains; (b) hairy appearance of spores. Bars, 1 μm.

and nutrient agar (Table 1). Diffusible pigments were observed in test media and melanin was produced on peptone-yeast extract-iron agar.

The cell wall of strain DAS-139^T contained LL-diaminopimelic acid and glycine as the diagnostic diamino acids, which indicated that it has a cell-wall of chemotype I (Lechevalier & Lechevalier, 1970a, b). No diagnostic sugars were detected in whole-cell hydrolysates. Phosphatidylethanolamine is a diagnostic phospholipid detected (phospholipid type II *sensu* Lechevalier *et al.*, 1977). The predominant menaquinones were MK-9(H₆) (76 %), MK-9(H₄) (14 %) and MK-9(H₈) (10 %). The major fatty acids found were iso-C_{16:0} (30 %), anteiso-C_{15:0} (16 %), iso-C_{15:0} (12 %), anteiso-C_{17:0} (9 %), iso-C_{16:1} (7 %) and iso-C_{14:0} (6 %). Thus, chemotaxonomic and phenotypic data showed that strain DAS-139^T should be assigned to the genus *Streptomyces*.

The almost-complete 16S rRNA gene sequence (1524 nt) of strain DAS-139^T was aligned manually with the corresponding partial 16S rRNA sequences of the type strains of representative members of the genus *Streptomyces* retrieved from the GenBank, EMBL and DDBJ databases by using BLAST (Altschul *et al.*, 1997). Phylogenetic analyses based on 16S rRNA gene sequences showed that the novel isolate falls into one distinct subclade with *Streptomyces scabiei* ATCC 49173^T (GenBank accession no. D63862) and *Streptomyces diastatochromogenes* ATCC 12309^T (D63867) with which it shared 16S rRNA gene sequence similarity of

Table 1. Culture characteristics of strain DAS-139^T on various ISP media

Colours were taken from ISCC-NBS Color Chart standard sample no. 2106 (Kelly, 1964). Plates were incubated for 3–4 weeks at 28 °C.

Medium	Growth	Sporulation	Diffusible pigment	Colony colour	
				Aerial mycelium	Substrate mycelium
Yeast extract agar (ISP 2)	Good	Good	Yellow	Yellow–brown	Grey–yellow
Oatmeal agar (ISP 3)	Good	Good	Brown	White	Reddish brown
Inorganic salts–starch agar (ISP 4)	Poor	Poor	Absent	White	Light yellow
Glycerol–asparagine agar (ISP 5)	Good	Good	Reddish brown	Light pink	Reddish brown
Tyrosine agar (ISP 7)	Good	Good	Black	Dark pink	Dark brown
Czapek's agar	Good	Moderate	Pale yellow	Pink	Dark reddish
Nutrient agar	Good	Moderate	Absent	Light brown	Grey–reddish

>99.0% (11 and 17 nucleotide differences out of 1524, respectively). The phylogenetic tree based on the 16S rRNA gene sequence of strain DAS-139^T and most closely related type strains of species of the genus *Streptomyces* is shown in Fig. 2. DNA–DNA relatedness tests were performed between strain DAS-139^T, *S. scabiei* ATCC 49173^T and *S. diastatochromogenes* ATCC 12309^T using the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992). The DNA–DNA relatedness values of strain DAS-139^T with *S. scabiei* ATCC 49173^T and *S. diastatochromogenes* ATCC 12309^T were 36% (36 ± 0.3) and 43.0%, respectively, and both values were significantly lower than 70%, the level considered to be the threshold value for the delineation of genomic species (Wayne *et al.*, 1987). Phenotypic comparisons were made between strain DAS-39^T and closely related species of the genus *Streptomyces* in order to differentiate strain DAS-139^T (Table 2). The G + C content of the DNA of strain DAS-139^T was 71.8 mol%. On the basis of morphological, chemotaxonomic and phylogenetic evidence and physiological and biochemical distinctiveness, it is suggested that strain DAS-139^T is a new member of the genus *Streptomyces*, for which the name *Streptomyces deccanensis* sp. nov. is proposed.

Description of *Streptomyces deccanensis* sp. nov.

Streptomyces deccanensis [dec.can.en'sis. N.L. masc. adj. *deccanensis* pertaining to Deccan Trap, India, the geographical region (Gulbarga) from where the type strain was isolated].

Gram-positive, aerobic, non-motile, alkali-tolerant and thermotolerant with good growth of hyphae without fragmentation. Long, hairy, oval-shaped spores are arranged in straight chains. Grows well on almost all media tested (mentioned in Table 1). Colonies are whitish to pink, smooth, powdery and 0.5–1.0 mm in diameter. Casein and cellulose are degraded. Positive in tests for nitrate reduction and gelatin liquefaction. Soluble pigments are observed in all test media and melanin is observed in peptone–yeast extract–iron agar. The temperature range for growth is 28–45 °C, with an optimum at 40 °C. Grows at pH 7–12 and in the presence of 7% NaCl. Good growth is found on almost all carbon sources tested, more detailed phenotypic properties are mentioned in Table 2. Cell wall contains LL-diaminopimelic acid and glycine. Whole-cell hydrolysates mainly contain galactose and glucose and no diagnostic sugars. Predominant menaquinones are MK-9(H₆) (76%),

Fig. 2. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA gene sequences, showing the position of strain DAS-139^T among its phylogenetic neighbours. Asterisks indicate branches that were recovered using least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) algorithms. Numbers at the branch nodes are bootstrap values, expressed as a percentage of 1000 replicates (only values above 50% are shown). Bar, 0.005 substitutions per nucleotide position.

Table 2. Morphological and phenotypic characteristics that differentiate strain DAS-139^T from closely related species of the genus *Streptomyces*

Taxa: 1, strain DAS-139^T; 2, *S. scabies* ATCC 49173^T; 3, *S. diastatochromogenes* ATCC 12309^T. +, Positive; –, negative; ND, not determined; R, red; OB, olive brown; GY, greyish yellow; SC, straight chains; RF, rectiflexibles. Data for reference strains were taken from Shirling & Gottlieb (1968a, b; 1969), Williams *et al.* (1983). All taxa were able to use the following as a carbon source: D-glucose, L-arabinose, sucrose, D-xylose, D-mannitol and D-fructose. All taxa were capable of degrading casein.

Characteristic	1	2	3
Colony morphology	White	Grey	Grey
Reverse side colour	R	OB	GY
Spore shape	Hairy	Smooth	Smooth
Spore chain	SC	RF	SC
Melanin	+	–	+
Soluble pigments	+	+	–
Degradation of:			
Cellulose	+	–	+
Pectin	–	–	ND
Starch	+	–	+
Growth at/with:			
NaCl (% w/v)	5.0	<4.0	<7.0
45 °C	+	–	+
pH	7–12	7–8	7.5
Carbon utilization:			
myo-Inositol	+	–	+
Raffinose	+	–	+
L-Rhamnose	+	–	+

MK-9(H₄) (14 %) and MK-9(H₈) (10 %). The diagnostic phospholipid is phosphatidylethanolamine. Major fatty acid components are C_{16:0} (29.78 %), anteiso-C_{15:0} (15.87 %), iso-C_{15:0} (11.97 %), anteiso-C_{17:0} (9.23 %), iso-C_{16:1} (7.24 %) and iso-C_{14:0} (5.88 %), iso-C_{17:1} (3.98), C_{16:0} (3.72) and iso-C_{17:0} (3.66). Grows optimally at 28 °C and pH 9.0–10.0. The DNA G + C content is 71.8 mol%.

The type strain, DAS-139^T (=KCTC 19241^T=CCTCC AA 207004^T), was isolated from a soil sample collected from Gulbarga, Karnataka Province, Deccan Trap, India.

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