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Full Length Research Paper

Streptomyces sannurensis sp. nov., a new alkaliphilic member of the genus *Streptomyces* isolated from Wadi Sannur in Egypt

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The taxonomic position of an actinomycete isolated from a soil sample collected from Wadi Sannur in Egypt was established using a polyphasic approach. The isolate, which was designated WS 51^T, was shown to have chemical and morphological properties typical of streptomycetes. An almost complete 16S rDNAgene sequence of the strain was generated and compared with corresponding sequences of representative streptomycetes. The resultant data confirmed the classification of the strain in the genus *Streptomyces* but also showed that it formed a distinct phyletic line within the 16S rDNA*Streptomyces* gene tree. The organism was most closely associated to the type strains of *Streptomyces hygroscopicus*, *Streptomyces malaysiensis* and *Streptomyces yatensis* but was readily separated from them using a range of phenotypic properties. It is proposed that strain WS 51^T (= CCTCC 001032^T = DSM 41834^T) be classified in the genus *Streptomyces* as *Streptomyces sannurensis* sp. nov.

Key words: *Streptomyces sannurensis* sp. nov., polyphasic taxonomy, alkaliphilic streptomycete.

INTRODUCTION

The genus *Streptomyces* encompasses aerobic, Gram-positive actinomycetes that form an extensively branched, substrate mycelium, aerial hyphae which typically differentiate into chains of spores, have LL-diaminopimelic acid but lack major characteristic sugars in whole-organism hydrolysates (wall chemotype 1 *sensu:* Lechevalier and Lechevalier, 1970) and contain DNA rich in guanine plus cytosine (Williams et al., 1989; Manfio et al., 1995). The taxon contains more than 600 validly described species, representatives of which have been assigned to lumpy groups based on 16S rDNAsequence data (Bull et al., 2005). It is evident that the genus *Streptomyces* remains underspeciated (Sembiring et al., 2000; Manfio et al., 2003) though several validly described species have become subjective synonyms of previously described species (Lanoot et al., 2002, 2004; Hatano et al., 2003; Kumar and Goodfellow, 2010). It is apparent that the description of *Streptomyces* species needs to be based on a combination of phenotypic and genotypic properties (Kim et al., 2004; Saintpierre-Bonaccio et al., 2004) and even on a multilocus phylogeny (Guo et al., 2008).

Novel streptomycetes are still in great demand as a source of new commercially significant bioactive compounds (Bérdy, 1995; Holtzel et al., 2003; Xu et al., 2004). In the course of a screening programme designed to isolate novel bioactive actinomycetes from environmental samples collected from Wadi Sannur in the Eastern Desert of Egypt, an actinomycete, designated WS 51, was isolated and provisionally assigned to the genus *Streptomyces*. The aim of the present study was to determine the taxonomic status of this isolate using

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genotypic and phenotypic procedures. The resultant data indicate that the organism should be classified as a new *Streptomyces* species for which the name *Streptomyces sannurensis* sp. nov. is proposed.

MATERIALS AND METHODS

Isolation and maintenance of the organism

Strain WS 51^T was isolated from a soil suspension used to inoculate a medium A agar plate which was incubated at 28°C for 14 days; the soil sample was collected from the Eastern Desert of Egypt at Wadi Sannur. The isolation medium, which had been recommended by Sato et al. (1983) for the isolation of alkalitolerant and alkaliphilic microorganisms, contained (g/L) glucose, 10.0; peptone, 5.0; yeast extract, 5.0; K_2HPO_4, 1.0; $MgSO_4$. $7H_20$, 0.2; Na_2CO_3, 10 g; agar, 15; distilled water, 1 L; the sodium carbonate was sterilised separately and added to the basal medium; the resultant preparation was adjusted to pH 10.5 using $NaHCO_3$-Na_2CO_3 buffer (Sambrook and Russell, 2001). A preliminary test was carried out to confirm its requirement for alkalinity. It was unable to grow below pH 7.0. The isolate was maintained on modified Bennett's agar (Jones, 1949) at pH 10.0 and as hyphae and spores in 20% glycerol at -20°C.

Morphology and cultural characters

The isolate was grown on medium A (Sato et al., 1983), Czapek's (Waksman, 1967), modified Bennett's (Jones, 1949), nutrient (Waksman, 1961) and ISP 1, 2, 3, 4, 5 and 7 (Shirling and Gottlieb, 1966) agar media, all adjusted to pH 10 and incubated for 14 days at 28°C. Following incubation for 28 days at 28°C, aerial spore mass colour, substrate mycelium colour and the colour of any diffusible pigments were recorded using the National Bureau of Standards (NBS) Colour Name Charts (Kelly, 1958; NBS, 1964). Spore chain morphology and spore surface ornamentation were examined by using light and scanning electron microscopes (JEOL, JSM-5600 LV) to study a culture grown on yeast extract-malt extract agar (ISP 2 medium; Shirling and Gottlieb, 1966) for 2 weeks at 28°C.

Chemotaxonomy

Biomass for the chemical analyses was prepared by growing the strain in shake flasks of medium A broth (Sato et al., 1983) at 200 rpm for 7 days at 28°C. Cultures were checked for purity, harvested by centrifugation, washed three times with distilled water and freeze-dried. Determination of the isomeric form of diaminopimelic acid and the whole-organism sugar was done as described by Hasegawa et al. (1983), and Staneck and Roberts (1974), respectively. Polar lipids and fatty acids were extracted and detected as previously described (Minnikin et al., 1977; Sutcliffe, 2000), while isoprenoid quinone were extracted, purified and identified by HPLC as described by Collins (1985).

Phenotypic tests

The organism was examined for a broad range of phenotypic tests using media and methods described by Williams et al. (1983); all media were adjusted to pH 10.0 and incubated at 28°C for 14 days.

16S rDNA sequencing and phylogenetic analysis

Biomass for the 16S rDNA sequencing study was prepared as described previously for chemotaxonomy. Genomic DNA was extracted from the biomass as described by Orsini and Romano-Spica (2001). PCR-mediated amplification of 16S rDNA, purification of the PCR products and sequencing of the products were carried out following the procedure of Cui et al. (2001). The resultant 16S rDNAgene sequence was aligned manually against available corresponding streptomycete sequences retrieved from the DDBJ, EMBL and GenBank databases. Phylogenetic trees were inferred for the test strain and its nearest neighbours using the least-squares (Fitch and Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou and Nei, 1987) tree-making algorithms from the PHYLIP suite of programs (Felsenstein, 1993). The distance model of Jukes and Cantor (1969) was used to generate an evolutionary distance matrix. The resultant tree topologies were evaluated by bootstrap analyses of the neighbour-joining method based on 1000 resamplings using the SEQBOOT and CONSENSE programs from the PHYLIP package (Felsenstein, 1993). The Clustal X program version 1.8 (Thompson et al., 1997) was used for the multiple alignment and the phylogenetic analyses.

DNA base composition

The DNA base composition of genomic DNA isolated from strain WS 51^T according to the procedure described by Hopwood et al. (1985) was determined using the thermal denaturation method (Mandel and Marmur, 1968) and a Shimadzu UV-visible spectrophotometer.

RESULTS AND DISCUSSION

There are some studies on the alkaliphilic actinomycetes (Mikami et al., 1982; Groth et al., 1997; Jones et al., 1998; Hozzein et al., 2004), few of them were on their applications, as production of alkaline enzymes (Horikoshi, 1999), bioactive substances (Tsujibo et al., 1990) and enzyme inhibitors (Bahn et al., 1998). But few reports were found on description of new species of the genus *Streptomyces* (Li et al., 2005; Dastager et al., 2008), which is very important for discovery of novel secondary metabolites.

The chemical and morphological properties of isolate WS 51^T were found to be consistent with its assignment to the genus *Streptomyces* (Williams et al., 1989; Manfio et al., 1995). The organism forms an extensively branched substrate mycelium, aerial hyphae that differentiate into chains of spores, contains the LL-isomer of diaminopimelic acid in the cell wall peptidoglycan, lacks major whole-organism sugars, contains phosphatidyl ethanolamine as a diagnostic polar lipid, MK-9 (H_6), MK-9 (H_2) and MK-9(H_8) as the major menaquinones, iso-C16:0, anteiso-C15:0, anteiso-C17:0, iso-C14:0 as the major fatty acid components, and has DNA rich in guanine plus cystosine (71.9 mol%).

The classification of the organism in the genus *Streptomyces* was underpinned by the nucleotide (nt) sequence data analysis which showed that it forms a distinct phyletic line in the 16S rDNA*Streptomyces* gene tree. Comparison of the almost complete 16S rDNAgene sequence of strain WS 51^T (1494 nt) with those of its

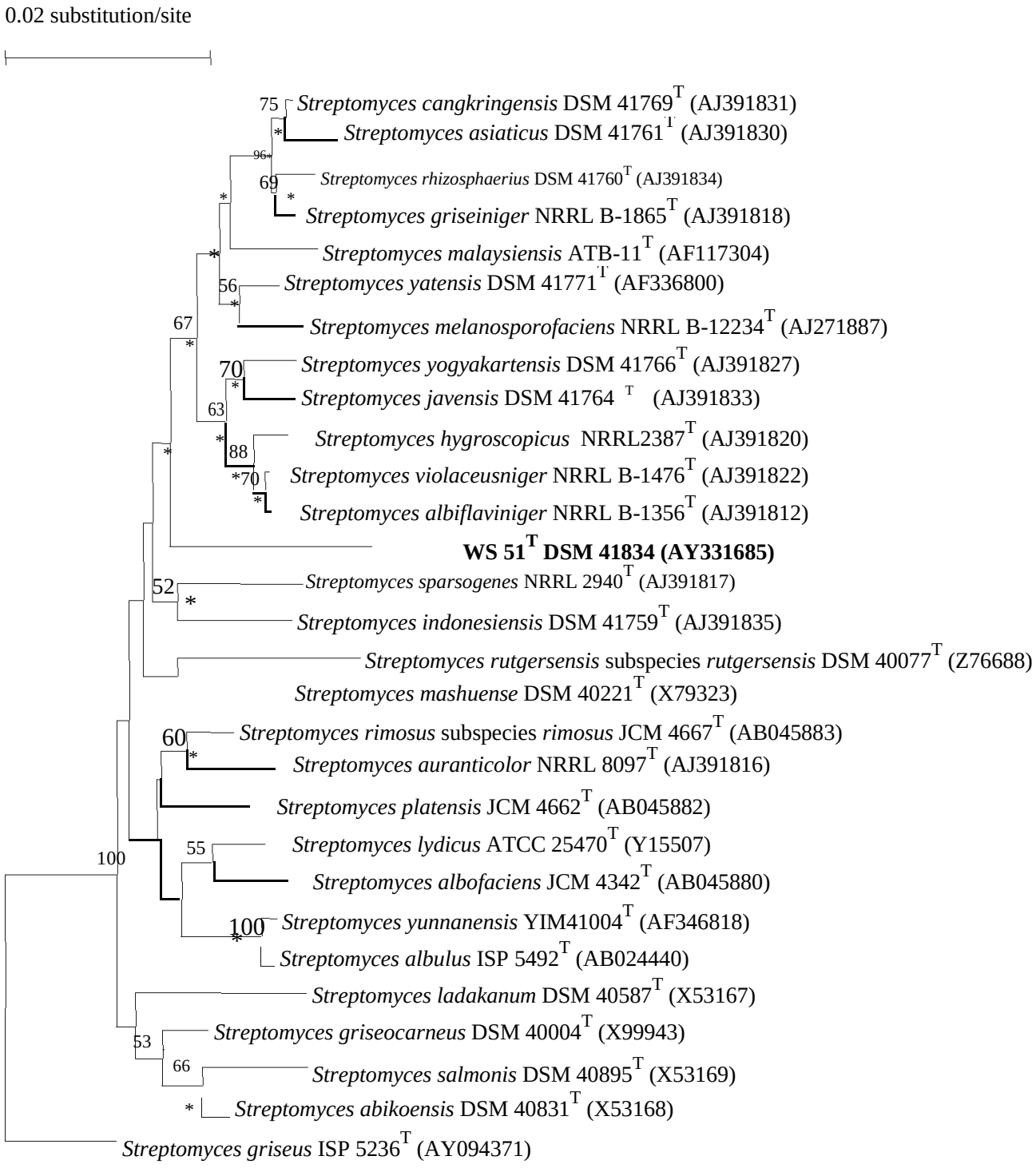

Figure 1. Neighbour-joining tree (Saitou and Nei, 1987) showing relationship between strain WS 51^T and its closest relatives; and representatives of the genus *Streptomyces* based on almost complete 16S rDNA sequences. The asterisks denote branches that were also recovered using the least-squares (Fitch and Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) treeing algorithms. The numbers at the nodes indicate the level of bootstrap support (%) based on a neighbour-joining analysis of 1000.

closest relatives and representatives of the genus *Streptomyces* is shown in Figure 1. The organism is most closely, but nevertheless loosely, associated with the type strains of *Streptomyces yatensis* (97.6%), *Streptomyces malaysiensis* (97.3%) and *Streptomyces hygroscopicus* (97.1%). DNA:DNA relatedness studies

Table 1. Characters distinguishing strain WS 51^T from the type strains of phylogenetically closely related *Streptomyces* species.

Character	Strain WS 51^T	*S. hygroscopicus* NRRL 2387^T	*S. malaysiensis* DSM 41697^T	*S. yatensis* DSM 41771^T
Morphology				
Spore chains	Long open looped	Spiral	Spiral	Spiral
Spore surface	Smooth	Warty	Rugose	Rugose
Degradation of				
Adenine	-	+	+	-
Hypoxanthine	-	+	+	+
Tyrosine	-	+	+	+
Enzyme activity				
H_2S production	-	-	-	+
Nitrate reduction	-	+	+	-
Growth at 45°C	+	+	-	-
Growth on sole carbon sources				
L(+) arabinose	-	+	+	-
meso-inositol	-	-	+	+
D(+) raffinose	+	-	+	+
Sodium citrate	-	+	-	-

+, Positive; -, negative. Data for the type strains of the established species were taken from Shirling and Gottlieb (1972), Al-Tai et al. (1999) and Saintpierre et al. (2004).

Table 2. Cultural characteristics of strain WS 51^T.

Medium [a]	Growth	Aerial spore mass	Substrate mycelium
Czapek's agar	Moderate	Light reddish brown	Reddish brown
Glycerol asparagine agar (ISP 5)	Good	Gray yellow pink	Deep reddish brown
Inorganic salts-starch agar (ISP 4)	None	None	None
Medium A agar	Abundant	White	Pale yellow
Modified Bennett's agar	Abundant	Yellowish white	Light yellow brown
Nutrient agar	Abundant	Yellowish pink	Light brown
Oatmeal agar (ISP 3)	Poor	None	Deep yellow brown
Tryptone yeast extract agar (ISP 1)	Moderate	White	Grayish yellow
Yeast extract-malt extract agar (ISP 2)	Good	Pinkish gray	Deep reddish brown

[a] All media were adjusted to pH 10 .0.

were not carried out between these strains as representatives of other *Streptomyces* species with similarly low 16S rDNAsequence similarities show DNA relatedness values well below 80% (Sembiring et al., 2000; Kim and Goodfellow, 2002; Manfio et al., 2003), the cut-off point recommended for the recognition of genomic species of *Streptomyces* (Labeda and Lyons, 1992; Labeda, 1993, 1998). Strain WS 51^T can also be distinguished from its three closest phylogenetic neighbours using a range of phenotypic properties, notably by using key morphological features (Table 1). Indeed, *S. hygroscopicus*, *S. malaysiensis* and *S. yatensis* all belong to the *Streptomyces violaceusniger* clade, a well circumscribed group of streptomycetes (Sembiring et al., 2000; Saintpierre et al., 2003). The cultural characteristics of the tested strain are shown in Table 2.

It is evident that strain WS 51^T can be distinguished from representatives of closely related validly described *Streptomyces* species using a combination of genotypic

and phenotypic properties. It is, therefore, proposed that this organism be given species status in the genus *Streptomyces* as *S. sannurensis* sp. nov.

Description of *S. sannurensis* sp. nov.

S. sannurensis (san.nur.en'sis. N.L. max. *sannurensis*) pertaining to Sannur, a valley (wadi) in the Eastern Desert of Egypt, the source of the isolate. Aerobic, non-acid fast, Gram-positive actinomycete which forms an extensively branched substrate mycelium that carries abundant aerial hyphae which differentiate into long, open-looped chains of smooth-surfaced spores. The colour of the aerial spore mass varies from white to yellowish white to reddish brown and that of the substrate mycelium from yellowish brown/grayish yellow to reddish brown. A yellowish brown soluble pigment is formed on modified Bennett's agar. It grows in condition of temperature ranging from 10 to 45°C and pH from 7.0 to 12.0, though growth is scant at the lowest temperature and pH values. It grows optimally at pH 9.5 to 10 and temperature range of 28 to 30°C.

Growth occurs in the presence of 5% sodium chloride. It degrades casein but not tyrosine, hypoxanthine adenine or tributyrin. Cellobiose, dulcitol (weak), D(+) fructose (weak), D(+) galactose (weak), D(+) glucose, D(+) lactose, D(+) mannose, D(+) mannitol, xylitol, sodium acetate (weak) and sodium succinate (weak) are used as sole carbon sources for energy and growth, but not *meso*-inositol; D(+) maltose, L(-) rhamnose, D(-) ribose, D(-) sorbitol or D(+) sucrose. Arginine, asparagine, cysteine (weak), glycine, histidine, methionine (weak), phenylalanine, serine, threonine, valine, sodium nitrate and potassium nitrate are used as sole nitrogen sources but not hydroxyproline. The whole-cell hydrolysate contains only LL-diaminopimelic acid, glucose and galactose. The major polar lipids are diphosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl inositol mannosides and unknown glucosamine-containing phospholipids. The menaquinone pattern consists of MK-9(H_6), MK-9(H_2) and MK-9(H_8). The DNA base composition is 71.9 mol%. The type and only strain is WS 51^T (= DSM 41834^T = CTCC AA001032^T).

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