Antonie van Leeuwenhoek (2008) 93:305–313
DOI 10.1007/s10482-007-9205-z

ORIGINAL PAPER

Streptomyces sudanensis sp. nov., a new pathogen isolated from patients with actinomycetoma

Erika T. Quintana · Katarzyna Wierzbicka · Pawel Mackiewicz · Abdalla Osman · Ahmed H. Fahal · Mohamed E. Hamid · Jolanta Zakrzewska-Czerwinska · Luis A. Maldonado · Michael Goodfellow

Received: 12 July 2007 / Accepted: 27 September 2007 / Published online: 22 December 2007

Abstract Nine strains isolated from mycetoma patients and received as *Streptomyces somaliensis* were the subject of a polyphasic taxonomic study. The organisms shared chemical markers consistent with their classification in the genus *Streptomyces* and formed two distinct monophyletic subclades in the *Streptomyces* 16S rRNA gene tree. The first subclade contained four organisms, including the type strain of *S. somaliensis*, and the second clade the remaining five strains which had almost identical 16S rRNA sequences. Members of the two subclades were sharply separated using DNA:DNA relatedness and phenotypic data which also showed that the subclade 1 strains formed an heterogeneous group. In contrast, the subclade 2 strains were assigned to a single genomic species and had identical phenotypic profiles. It is evident from these data that the subclade 2 strains should be recognised as a new species of *Streptomyces*. The name proposed for this new species

Erika T. Quintana and Katarzyna Wierzbicka contributed equally to this work.

The GenBank accession numbers for the 16S rRNA gene sequences of *Streptomyces somaliensis* DSM 40738^T and *Streptomyces sudanensis* DSM 41607, DSM 41608, DSM 41609, SD 504^T and SD 509 are EF540897, EF540898, EF540999, EF515876 and EF540900.

E. T. Quintana · L. A. Maldonado · M. Goodfellow (✉)
School of Biology, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK
e-mail: m.goodfellow@ncl.ac.uk

Present Address:
E. T. Quintana
Instituto Politécnico Nacional (IPN), Centro Interdisciplinario de Investigaciones y Estudios sobre Medio Ambiente y Desarrollo (CIIEMAD), CP 07340 Mexico, DF, Mexico

K. Wierzbicka · J. Zakrzewska-Czerwinska
Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wroclaw, Poland

P. Mackiewicz
Faculty of Biotechnology, Department of Genomics, University of Wrocław, Przybyszewskiego 63/77, 51-148 Wroclaw, Poland

A. Osman · A. H. Fahal
Faculty of Medicine, Mycetoma Research Centre, University of Khartoum, PO Box 102, Khartoum, Sudan

M. E. Hamid
Faculty of Veterinary Science, Department of Preventive Medicine and Public Health, University of Khartoum, Khartoum North, Sudan

Present Address:
L. A. Maldonado
Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México (UNAM), CP 04510 Mexico, DF, Mexico

is *Streptomyces sudanensis* sp. nov. The type strain is SD 504^T (DSM = 41923^T = NRRL B-24575^T).

Keywords Mycetoma · Polyphasic taxonomy · Streptomyces

Introduction

Most aerobic filamentous actinomycetes are soil saprophytes but some are serious pathogens of man. The latter include organisms that cause actinomycetoma, a localised chronic, destructive and progressive infection of skin, subcutaneous tissue and eventually bone (McNeil and Brown 1994; Develoux et al. 1999). This disease is endemic in certain tropical and subtropical regions where it has a devastating effect on patients as it frequently leads to deformities, disabilities and eventually amputation of the affected organs. The main causal agents are considered to be *Actinomadura madurae*, *Actinomadura pelletieri*, *Nocardia brasiliensis*, *Nocardia otitidiscaviarum*, *Nocardia transvalensis* and *Streptomyces somaliensis* (Trujillo and Goodfellow 2003).

Actinomycetoma is a major health problem in parts of Sudan (Mahgoub 1985; Fahal and Hassan 1992; Fahal 2004, 2006) where most cases are attributed to *S. somaliensis* (Gumaa and Mahgoub 1975; Taha 1983; Gumaa 1994; Fahal 2006). The causal agents of the disease still tend to be identified by the colour of grains (macroscopic pigmented colonies of the causal organism surrounded by masses of inflammatory cells) discharged from sinuses or from biopsy material and by the morphological characteristics of strains growing on standard cultivation media. Such practices, while understandable, are inappropriate for the accurate classification and identification of the causal organisms. It seems likely that patients who do not respond to standard therapeutic treatment may be infected with unknown actinomycetes and thereby require specific antibiotic treatment regimes. This is a serious problem as actinomycetoma becomes dangerous to health, or even life, when treatment is inadequate or delayed. A thorough microbiological diagnosis while desirable is not always possible due to difficulties in isolating and characterising the causal agents. A co-ordinated approach from the collection of specimens through to the identification of the causal agents of actinomycetoma has been described by Goodfellow (1996).

It is evident from the application of genotypic and phenotypic procedures that the genus *Streptomyces* as a whole is underspeciated (Manfio et al. 2003; Saintpierre-Bonaccio et al. 2004; Xu et al. 2006) though molecular systematic data show that some established species described using phenotypic criteria should be reduced to synonyms of previously described species (Lanoot et al. 2004, 2005; Liu et al. 2005). Members of the genus are generally considered to be non-pathogenic but the taxon does encompass pathogenic species, notably *Streptomyces scabies* and *Streptomyces somaliensis* (Williams et al. 1989; Bouchek-Mechiche et al. 2006). The present study was designed to clarify the taxonomy of actinomycetes isolated from mycetoma patients in the Sudan and presumptively identified as *S. somaliensis*. It was evident from the resultant polyphasic taxonomic study that some of these organisms belong to a new species of *Streptomyces*. The name proposed for this taxon is *Streptomyces sudanensis* sp. nov.

Materials and methods

Organisms and cultural conditions

The sources and strain histories of the organisms are given in Table 1. The SD coded strains were isolated on Sabouraud dextrose agar plates (MacFaddin 1985) which had been incubated at 37°C following inoculation with grains taken from deep excision biopsy material then washed several times in normal saline to remove debris and blood. The isolation plates were examined daily until actinomycete colonies were evident; the colonies were subcultured onto fresh Sabouraud dextrose agar plates and incubated at 37°C for 7 days. Biomass for the chemotaxonomic studies and for DNA isolation was obtained by growing each of the strains in 250 ml shake flasks (150 revolutions per minute) containing 50 ml^{-1} of either glucose–yeast extract broth (Gordon and Mihm 1962) or modified Sauton's broth (Mordarska et al. 1972), respectively at 28°C for 7 days. The biomass for the chemical analyses was washed twice in distilled water then freeze-dried, and that for the molecular systematic studies in NaCl–EDTA (0.1 M EDTA, pH 8.0, 0.1 M NaCl) prior to storage at −20°C.

Table 1 Sources and strain histories

DSM 40738^T	*S. somaliensis*; R.E. Gordon, Rutgers University, New Brunswick, USA; F. Mariat, IP 733; mycetoma of the foot of a 20-year old male
DSM 41607	*S. somaliensis*; F. Mariat, Institut Pasteur, Paris, France; IP 314; London School of Hygiene and Tropical Medicine, A-310
DSM 41608	*S. somaliensis*, D. Mackenzie, Mycological Reference Laboratory, Bristol, England, NCPF 1069; Khartoum, Sudan in 1970
DSM 41609	*S. somaliensis*, D. Mackenzie, NCPF 1070; Khartoum, Sudan in 1970
DSM 41610	*S. somaliensis*, D. Mackenzie, NCPF 1220; Khartoum, Sudan in 1970
SD 504	*S. somaliensis*; A.H. Fahal, Mycetoma Research Centre, University of Khartoum, Khartoum; white grain from mycetoma with sinuses on the right foot of a 20-year old pregnant woman from Kordofan, Western Sudan, 1999
SD 509	*S. somaliensis*; A.H. Fahal, white grain from mycetoma on the right foot of a 42-year old man from El Rahad, Kordofan, Western Sudan, 1999
SD 511	*S. somaliensis*; M.E. Hamid, Department of Preventive Medicine and Public Health, University of Khartoum, Khartoum, Sudan; mycetoma, 1999
SD 534	*S. somaliensis*; M.E. Hamid; mycetoma, 1999

DSM, Deutsche Sammlung von Mikroorganismen, Mascheroder Weg 1b, D-38124, Braunschweig, Germany

Chemotaxonomic and phenotypic properties

Standard chromatographic procedures were used to determine the isomers of diaminopimelic acid (Staneck and Roberts 1974) and the menaquinone composition (Collins 1994) of the test strains and appropriate marker organisms; the presence of mycolic acids were sought using an alkaline methanolysis procedure (Minnikin et al. 1980). The strains were examined for an extensive range of phenotypic properties using a standard inoculum (McFarland turbidity 5 in ¼ strength Ringer's solution) with media and methods described by Williams et al. (1983). They were also examined for their ability to grow on tryptone–yeast extract, yeast–malt extract, inorganic salts–starch and glycerol asparagine agars, that is, on ISP media 1, 2, 4 and 5, after Shirling and Gottlieb (1966).

16S rRNA gene sequencing analyses

Isolation of chromosomal DNA, PCR amplification and direct sequencing of 16S rRNA genes of the test strains was carried out as described previously (Kim et al. 1998). The resultant almost complete 16S rRNA sequences were compared with corresponding sequences of members of the genus *Streptomyces* obtained from GenBank (http://www.ncbi.nlm.nih.gov). The 16S rRNA secondary structure models were downloaded as DCSE files from the European Ribosomal RNA Database (http://www.psb.ugent.be/rRNA/). The sequences were aligned using CLUSTAL X 1.83 software (Thompson et al. 1997). Sites suitable for further phylogenetic analyses were extracted from the alignments using Gblocks 0.91b (Castresana 2000) assuming less stringent criteria.

Two phylogenetic analyses were carried out. In the first one, standard methods assuming independent substitutions in sites were used while the second one involved the use of methods that consider RNA secondary structure constraints and assume a correlation between substitutions in paired regions of RNA helices. In the first method phylogenetic trees were constructed by using five tree-making algorithms, neighbor-joining (NJ), minimum-evolution (ME), weighted least-squares (WLS), maximum-parsimony (MP) and maximum-likelihood (ML), from the Paup* 4.0b program (Swofford 1998). In the case of the ME, WLS, MP and ML methods, the trees were searched from 10 starting trees obtained by stepwise addition with random-addition sequences, followed by the use of the TBR branch-swapping algorithm on each starting tree; bootstrap analyses were performed on 2,000 replicates for each of the methods. The MrBayes 3.1.2 program (Ronquist and Huelsenbeck 2003) was used to find the Bayesian tree by application of two independent runs and 7 Markov chains. Trees were sampled every 100 generations of 40 million generations and the first 25,266,000

generations discarded. In the distance, maximum-likelihood and Bayesian analyses, the GTR+I+Γ model was applied using the Modeltest 3.7 program (Posada and Crandall 1998). In the MP analysis, transversions were weighted two times transitions.

Three programs were used in the second approach which considered the secondary structure of RNA genes. In inferring the ML tree by TREEFINDER (Jobb et al. 2004) the bactSLT+I+Γ model of nucleotide substitutions and 2,000 replicates in the bootstrap analysis were applied. In searching for a tree using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) two independent runs and 7 Markov chains were carried out. The doublet GTR+I+Γ model for stem regions of rRNA sequences and the simple GTR+I+Γ model for loops were used. Trees were sampled every 100 generations out of 20 million generations and the first 11,246,000 generations discarded. In the case of PHASE (Jow et al. 2002) the paired-site RNA16A+I+Γ model for stems and the simple GTR+I+Γ model for loops were used. This analysis ran through 2 million initial generations, the trees were sampled every 100 generations during the additional 1 million generations. The 50,000 sampled trees generated from the five independent runs were concatenated to produce the consensus tree (Fig. 1).

DNA base composition

Chromosomal DNA was extracted from wet biomass of *S. somaliensis* DSM 40738^T and SD 504^T after Mordarski et al. (1976). The thermal denaturation procedure of Marmur and Doty (1962) was used to determine the guanine (G) plus cystosine (C) content of the DNA preparations, using the following equation: mol.% G+C = (Tm −53.9) 2.44, where Tm is the melting temperature. The preparations were examined either three or four times in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M − trisodium citrate, pH 7.0) with DNA taken from *Streptomyces avermitilis* MA-4680 (70.7 mol.% GC) used as the control.

DNA:DNA relatedness studies

Purified DNA was prepared from all of the test strains following the procedure described by Mordarski et al. (1976). The DNA:DNA relatedness studies were carried out using the nitrocellulose filter method, as described by Kim et al. (1998), using probes prepared from strains DSM 40738^T, DSM 41607, DSM 41608, DSM 41609, SD 504^T, SD 509 and SD 511. The experiments were performed at least three times under optimal hybridization conditions. The amount of bound probe DNA was estimated by scintillation counting and relatedness values expressed as the percentage of probe bound (mean of hybridization assays) relative to the corresponding homologous reactions.

Results

16S rRNA gene sequences

Comparison of the nearly complete 16S rRNA gene sequences of the strains (1,363–1,521 nucleotides) with corresponding sequences of streptomycete type strains available from GenBank confirmed that all of the tested strains belong to the genus *Streptomyces* (data not shown). It is apparent from Fig. 1 that the nine strains fall into two subclades which are sharply separated from one another and from representatives of phylogenetically related taxa. The monophyly of each of the two subclades is supported by all of the tree-making algorithms and by high bootstrap values.

Subclade 1 encompasses four organisms, *Streptomyces somaliensis* DSM 40738^T, DSM 41610, SD 511 and SD 534, which form a relatively heterogeneous taxon. The two most closely related organisms, *S. somaliensis* DSM 40738^T and DSM 41610, share a 16S rRNA gene sequence similarity of 99.9%. The type strain shares lower sequence similarities with strains SD 511 and SD 534, namely 99.3% and 99.0%, respectively. In turn, strains SD 511 and SD 534 have the lowest sequence similarity, 98.5%, in common. In contrast, the five organisms which formed subclade 2, strains DSM 41607, DSM 41608, DSM 41609, SD 504 and SD 509, have 16S rRNA sequence similarities within the range 99.8–99.9%. The highest sequence similarity found between *S. somaliensis* DSM 40738^T and a member of the second subclade is with strain DSM 41607; these organisms shared a 16S rRNA sequence similarity of 97.6%.

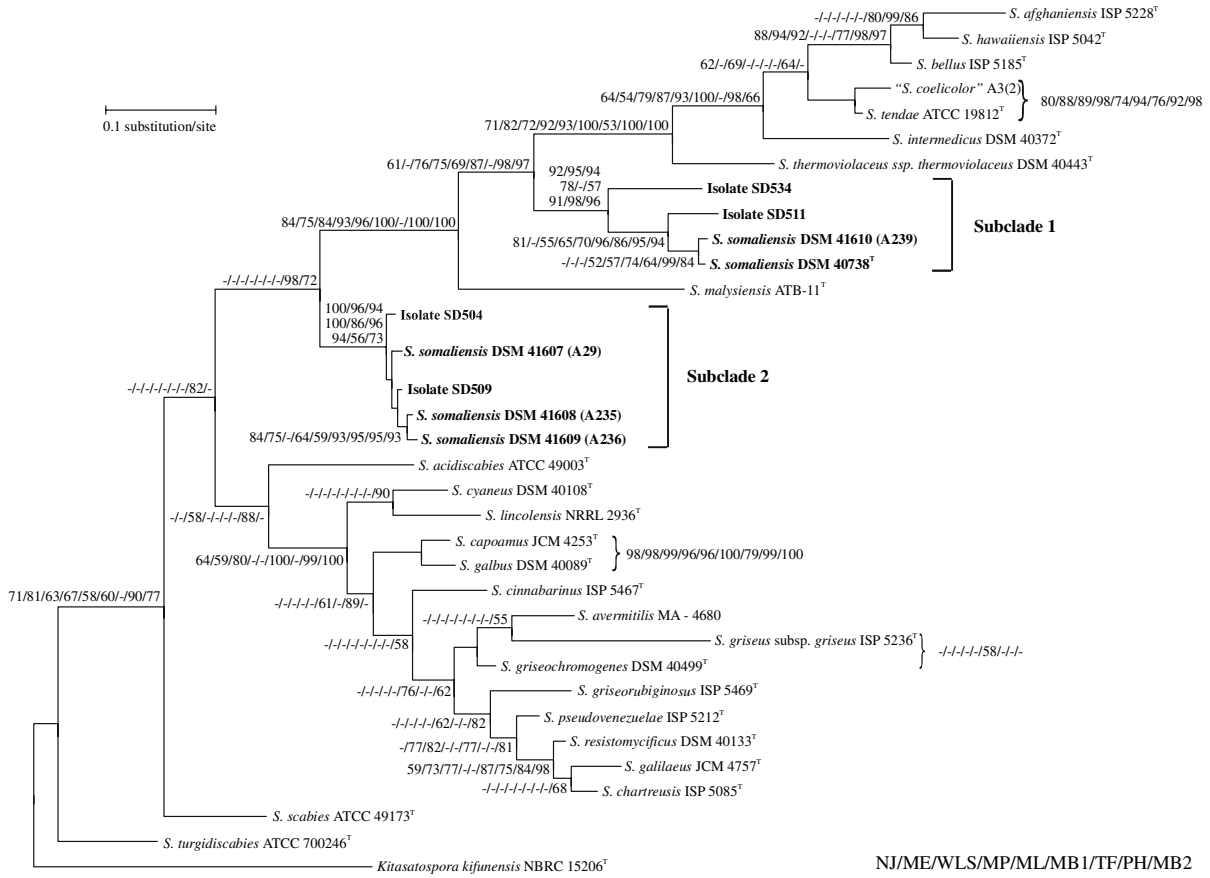

Fig. 1 Phylogenetic tree based on 16S rRNA sequences (1,563 sites) of 34 representatives of the family *Streptomycetaceae*. The tree was constructed using a Bayesian approach based on the MrBayes 3.1.2 program that considers RNA secondary structure constraints. The doublet GTR+I+Γ model was assumed for the stem regions of the rRNA sequences and the simple GTR+I+Γ model was applied for the loops. The numbers at the nodes in the order shown correspond to bootstrap percentages or posterior probabilities (in the case of the Bayesian approach: MB1, MB2, PH) based on (i) standard phylogenetic methods without RNA secondary structure constraints, namely the neighbor-joining (NJ), minimum-evolution (ME), weighted least-squares (WLS), maximum-parsimony (MP) and maximum-likelihood (ML) tree-making algorithms, Bayesian method by MrBayes 3.1.2 (MB1) and (ii) methods considering RNA secondary structure constraints: maximum-likelihood by TREEFINDER (TF), Bayesian method by PHASE (PH) and by MrBayes 3.1.2 (MB2). Bootstrap percentages and posterior probabilities lower or equal to 50% were omitted or are indicated by a dash "-" (Felsenstein 1985)

DNA:DNA relatedness studies

It can be seen from Table 2 that the five subclade 2 strains share DNA:DNA relatedness values between 70% and 100% using probe DNA from each of the five strains. It is evident that the mean DNA:DNA relatedness values for each of the reciprocal crosses is above the recommended 70% cut-off point (Wayne et al. 1987). It can, therefore, be concluded that strains DSM 41607, DSM 41608, DSM 41609, SD 504 and SD 509 belong to a single genomic species. In contrast, *S. somaliensis* DSM 40738^T is relatively closely related to strain SD 511. It is also apparent from Table 2 that the members of the two subclades share DNA:DNA relatedness values within the range of 16–65%.

Morphological, cultural, chemical and phenotypic characteristics

All of the tested strains had phenotypic properties consistent with their assignment to the genus *Streptomyces* (Williams et al. 1989; Manfio et al. 1995).

Table 2 DNA:DNA relatedness values between the tested strains

Source of unlabelled DNA	DNA binding (%)						
	Source of ^{3}H labelled DNA						
	DSM 40738^{T}	SD 511	DSM 41607	DSM 41608	DSM 41609	SD 504	SD 509
DSM 40738^{T}	*100*	*70* (19)	51	33	49	49 (7)	40
DSM 41610	57 (7)	33 (5)	30	21	31	29 (8)	32 (11)
SD 511	*77 (16)*	*100*	65	46	62	55 (7)	45 (22)
SD 534	21 (4)	29 (9)	23	16	23	25 (8)	17 (10)
DSM 41607	52 (15)	44 (4)	*100*	*81*	*86*	*100*	*80 (5)*
DSM 41608	51	45	*100*	*100*	*100*	*100*	*100*
DSM 41609	61 (11)	56 (3)	*81*	*80*	*100*	*98 (3)*	*78*
SD 504	47 (2)	45 (11)	*70*	*100*	*79*	*100*	*84 (1)*
SD 509	65 (3)	44	*99*	*70*	*100*	*100*	*100*
"S. coelicolor" A3(2)	9 (6)	10	14	6	8	17 (8)	15

^{T} , type strain. Standard deviations are given in brackets for the assays that were repeated several times (>3). Strains showing DNA relatedness values of 70% or more are given in italics

The organisms are aerobic, Gram-positive actinomycetes which form extensively branched substrate mycelia, have whole-organism hydrolysates rich in LL-diaminopimelic acid, lack mycolic acids, and have hexahydrogenated menaquinones with nine isoprene units as the predominant isoprenologue (52.2–52.3% of the total menaquinone composition), the corresponding octahydrogenated component forms the balance of the menaquinones. None of the organisms formed aerial hyphae on glycerol–asparagine, inorganic salts–starch, tryptone–yeast extract or yeast–malt extract agars. These results are in line with those from previous studies on *S. somaliensis* strains but nevertheless are unusual as members of the genus *Streptomyces* typically form an aerial spore mass (Williams et al. 1983, 1989). The mean DNA base composition of strain DSM 40378^{T} and SD 504^{T} are 70.5 and 70.1 mol.%, respectively.

Discussion

The strains received as *S. somaliensis* formed two distinct subclades in the 16S rRNA gene tree. The four strains assigned to subclade 1, which include the type strain of *S. somaliensis*, constituted a relatively heterogeneous group whereas the five subclade 2 organisms shared very high 16S rRNA gene similarities. DNA:DNA relatedness studies are used routinely to resolve the finer relationships between streptomycetes which share virtually identical 16S rRNA gene similarities, as exemplified by studies on representatives of the *S. griseus* (Liu et al. 2005) and *S. violaceusniger* (Goodfellow et al. 2007) subclades. The minimal level of DNA relatedness between strains required to circumscribe a genomic species is recommended as 70% (Wayne et al. 1987) though extensive DNA:DNA studies on *Streptomyces* strains implied that genomic relatedness values above 80% may correspond to species level relatedness in this genus (Labeda 1993, 1998; Labeda and Lyons 1992). It is evident from these recommended guidelines that the subclade 2 strains belong to a single genomic species.

Good congruence was found between the molecular systematic and corresponding phenotypic data. This is particularly so with the subclade 2 strains as these organisms shared identical phenotypic properties, some of which readily distinguished them from the strains assigned to subclade 1. It is evident from the combined genotypic and phenotypic data that the subclade 2 strains, that is, the strains received as *S. somaliensis* DSM 41607, DSM 41608, DSM 41609, SD 504^{T} and SD 509 should be recognised as a new species in the genus *Streptomyces*. The name proposed for this taxon is *Streptomyces sudanensis*.

Table 3 Phenotypic properties that distinguish members of subclade 2 from those assigned to subclade 1

Strain numbers	Subclade 1				Subclade 2
	DSM 40738^T	DSM 41610	SD 511	SD 534	DSM 41607–DSM 41609, SD 504^T, SD 509
Morphological properties on oatmeal agar					
Substrate mycelia colour	Light yellow	Light yellow	Light yellow	Yellow	Light yellow
API ZYM test					
Production of β-glucuronidase	+	−	−	−	−
Biochemical test					
Aesculin hydrolysis	+	−	−	+	−
Degradation of (% [w/v])					
Adenine (0.4)	+	−	−	+	−
Casein (0.5)	+	−	+	−	+
Hypoxanthine (0.4)	+	−	−	+	−
Growth on sole carbon sources (1%, w/v)					
Adonitol	+	+	−	+	+
Glycerol	+	+	−	+	+
Glycogen	+	+	−	+	+
meso-Inositol	+	+	−	+	+
D-raffinose	+	+	−	+	+
L-rhamnose	+	+	−	+	−
D-turanose	+	+	−	+	+
D-xylose	+	+	−	+	+
Growth on sole nitrogen sources (0.1%, w/v)					
L-aspartic acid	+	−	−	−	−
Growth at					
50°C	−	−	+	+	−
pH 11.0	−	−	+	+	−
Resistance to antibiotics ($\mu g\ ml^{-1}$)					
Clindamycin (2)	+	−	−	+	−
Gentamicin sulphate (10)	+	−	−	+	−
Streptomycin sulphate (10)	+	−	+	+	−

+, positive; −, negative; v, variable. All of the strains degraded elastin (0.3), gelatin (0.4) and tyrosine (0.4); grew between 15 and 45°C and from pH 5.0 to 10; used L-arabinose, D- and L-arabitol, arbutin, D-cellobiose, dextran, dulcitol, *meso*-erythritol, D-fructose, D-fucose, D-glucose, inulin, α-lactose, D-maltose, D-mannitol, D-melibiose, D-melezitose, D-ribose, D-sorbitol, L-sorbose, D-sucrose, starch and xylitol as sole carbon sources for energy and growth (at 1%, w/v), and L-arginine, L-alanine, L-α-aminobutyric acid, L-asparagine, L-aspartic acid, L-glutamic acid, L-lysine, L-histidine, L-*iso*leucine, L-leucine, L-lysine, L-methionine, DL-norleucine, DL-norvaline, L-ornithine, L-phenylalanine, L-proline, DL-serine, L-threonine and L-tryptophane as sole nitrogen sources (at 0.1% w/v) and were resistant to amoxicillin (30), colistin sulphate (25) and penicillin G (0.6 units ml^{-1}). In contrast, none of the organisms produced aerial hyphae: degraded tributyrin or xanthine (0.4); grew at 10°C, reduced nitrate, produced urea; or grew in the presence of ampicillin (30), cephaloridine (30), cephalexin (30), erythromycin (5), fusidic acid (10), novobiocin (5) or tetracycline hydrochloride (25)

Description of *Streptomyces sudanensis* sp. nov. (su.dan.en'sis. N.L. masc. adj. *sudanensis*, pertaining to the Sudan, the source of the isolates).

Aerobic, Gram-positive, non-acid-alcohol-fast, non-motile actinomycetes which form an extensively branched, light yellow substrate mycelium on glycerol–

asparagine, inorganic salts–starch, tryptone–yeast extract and yeast–malt extract agars. Neither aerial hyphae nor diffusible pigments are formed on these media. The organism has an oxidative type of metabolism, grows between 15 and 45°C and from pH 5.0 to 10.0. Aesculin and urease reactions are negative, nitrate is not reduced. Aesculin, L-fucose, D-galactose, D-mannose, pectin, D-salicin and D-trehalose are used as sole carbon sources for energy and growth (all at 1%, w/v). Grows in the presence of filter paper discs soaked in bacitracin (0.1 units ml^{-1}), penicillin G (1 unit ml^{-1}) and sulphamethoxazole (25 μg ml^{-1}), but not in the presence of ampicillin (30 μg ml^{-1}), cephalexin (30 μg ml^{-1}), cephaloridine (30 μg ml^{-1}), clindamicin (2 μg ml^{-1}), erythromycin (5 μg ml^{-1}), fusidic acid (10 μg ml^{-1}), gentamicin sulphate (10 μg ml^{-1}), streptomycin sulphate (10 μg ml^{-1}), tetracycline hydrochloride (25 μg ml^{-1}) or novobiocin (5 μg ml^{-1}). Additional phenotypic properties are shown in Table 3. The mean DNA base composition of SD 504^T is 70.1% G+C.

Isolated from patients with mycetoma infections in the Sudan. Type strain is SD 504^T (= DSM 41923^T = NRRL B-24575^T).

The present data show that mycetoma in the Sudan can be caused by at least two *Streptomyces* species the members of which show differences in antibiotic sensitivity patterns. However, it seems highly likely that the strains assigned to subcluster 1 belong to more than one species. The type strain of *S. somaliensis* and strain SD 511 share high DNA:DNA relatedness values and may belong to the same genomic species, but *S. somaliensis* SD 511 shows relatively low DNA gene similarities with these strains and hence belongs to a different genomic species. The phenotypic data show that the four subclade 1 strains have distinct phenotypic profiles. Further comparative taxonomic studies are needed to clarify the taxonomic relationships between the subclade 1 strains.

The results of the present study are in line with those from previous investigations which indicated that *S. somaliensis* strains form a heterogeneous group (Trujillo and Goodfellow 2003). These findings underline the importance of isolating and characterising the causal agents of actinomycetoma in an appropriate clinical setting as it is evident that the colour and texture of grains isolated from biopsy material is not a sufficiently rigorous practice for the accurate identification of streptomycetes that cause mycetoma.

Acknowledgements Erika T. Quintana and Luis A. Maldonado were supported by studentships from the Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico City, Mexico. Mohamed E. Hamid gratefully acknowledges receipt of a Wellcome Trust Research Development Award in Tropical Medicine.

References

Bouchek-Mechiche K, Gardan I, Andrivon D, Normand P (2006) *Streptomyces turgidiscabies* and *Streptomyces reticuliscabiei*: one genomic species, two pathogenic groups. Int J Syst Evol Microbiol 56:2771–2776

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17:540–552

Collins MD (1994) Isoprenoid quinones. In: Goodfellow M, O'Donnell AG (eds) Chemical methods in prokaryotic systematics. Academic Press Inc., London, pp 265–309

Develoux M, Dieng MT, Ndiaye B (1999) Mycetoma of the neck and scalp in Dakar. J Mycol Med 9:179–209

Fahal AH (2004) Mycetoma a thorn in the flesh. Trans R Soc Trop Med Hyg 98:3–11

Fahal AH (2006) Mycetoma: clinicopathological monograph. Khartoum University Press, Khartoum

Fahal AH, Hasan MA (1992) Mycetoma. Br J Surg 79: 1138–1141

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Goodfellow M (1996) Actinomycetes: *Actinomyces, Actinomadura, Nocardia, Streptomyces* and related genera. In: Collee JG, Fraser AG, Marmion BP, Simmons A (eds) Mackie and McCartney practical medical microbiology. Churchill Livingstone, Edinburgh, pp 343–359

Goodfellow M, Kumar Y, Labeda DP, Sembiring L (2007) The *Streptomyces violaceusniger* clade: a home for streptomycetes with rugose ornamented spores. Antonie van Leeuwenhoek 92:173–199

Gordon RE, Mihm JM (1962) Identification of *Nocardia caviae* (Erikson) nov. comb. Ann NY Acad Sci 98:628–636

Gumaa SA (1994) The aetiology and epidemiology of mycetoma. Sud Med J 32(suppl):14–22

Gumaa SA, Mahgoub ES (1975) Counterimmunoelectrophoresis in the diagnosis of mycetoma and its sensitivity as compared to immunodiffusion. Sabouradia 13:309–315

Jobb G, von Haeseler A, Strimmer K (2004) TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. BMC Evol Biol 4:18

Jow H, Hudelot C, Rattray M, Higgs P (2002) Bayesian phylogenetics using an RNA substitution model applied to early mammalian evolution. Mol Biol Evol 19: 1591–1601

Kim SB, Falconer C, Williams E, Goodfellow M (1998) *Streptomyces thermocarboxydovorans* sp. nov. and

Streptomyces thermocarboxydus sp. nov., two moderately thermophilic carboxydotrophic species from soil. Int J Syst Bacteriol 48:59–68

Labeda DP (1993) DNA relatedness among strains of the *Streptomyces lavendulae* phenetic cluster group. Int J Syst Bacteriol 43:822–825

Labeda DP (1998) DNA relatedness among the *Streptomyces fulvissimus* and *Streptomyces griseoviridis* phenotypic cluster groups. Int J Syst Bacteriol 48: 829–832

Labeda DP, Lyons AJ (1992) DNA relatedness among strains of the sweet potato pathogen *Streptomyces ipomoea* (Person and Martin 1949) Waksman and Henrici 1948. Appl Environ Microbiol 58:532–535

Lanoot B, Vancanneyt M, Dawyndt P, Crockaert M, Zhang J, Huang Y, Liu Z, Swings J (2004) BOX-PCR fingerprinting as a powerful tool to reveal synonymous names in the genus *Streptomyces*. Emended descriptions are proposed for the species *Streptomyces cinereorectus*, *S. fradiae*, *S. tricolor*, *S. columbiensis*, *S. filamentosus*, *S. vinaceus* and *S. phaeopurpureus*. Syst Appl Microbiol 27:84–92

Lanoot M, Vancanneyt M, Van Schoor A, Liu Z, Swings J (2005) Reclassification of *Streptomyces nigrifaciens* as a later synonym of *Streptomyces flavovirens*; *Streptomyces citreofluorescens*, *Streptomyces chrysomallus* subsp. *chrysomallus* and *Streptomyces fluorescens* as later synonyms of *Streptomyces anulatus*; *Streptomyces chibaensis* as a later synonym of *Streptomyces corchorusii*; *Streptomyces flaviscleroticus* as a later synonym of *Streptomyces minutiscleroticus*; and *Streptomyces lipmanii*, *Streptomyces griseus* subsp. *alpha*, *Streptomyces griseus* subsp. *cretosus* and *Streptomyces willmorei* as later synonyms of *Streptomyces microflavus*. Int J Syst Evol Microbiol 55:729–731

Liu Z, Shi Y, Zhang Y, Zhou Z, Lu Z, Li W, Rodriguez C, Goodfellow M (2005) Classification of *Streptomyces griseus* (Krainsky 1914) Waksman and Henrici 1948 and related species and the transfer of '*Microstreptospora cinerea*' to the genus *Streptomyces* as *Streptomyces yanii* sp. nov. Int J Syst Evol Microbiol 55:1605–1610

MacFaddin JF (1985) Media for isolation–cultivation–identification–maintenance of medical bacteria, vol 1. Williams and Wilkins, Baltimore

Mahgoub ES (1985) Mycetoma. Int J Dermatol 24:230–239

Manfio GP, Zakrzewska-Czerwinska J, Atalan E, Goodfellow M (1995) Towards minimal standards for the description of *Streptomyces* species. Biotechnologia 7–8:242–253

Manfio GP, Atalan E, Zakrzewska-Czerwinska J, Mordarski M, Rodriguez C, Collins MD, Goodfellow M (2003) Classification of novel soil streptomycetes as *Streptomyces aureus* sp. nov., *Streptomyces laceyi* sp. nov. and *Streptomyces sanglieri* sp. nov. Antonie van Leeuwenhoek 83:245–255

Marmur J, Doty P (1962) Determination of base composition of deoxyribonucleic acid from its denaturation temperature. J Mol Biol 5:109–118

McNeil MM, Brown JM (1994) The medically important aerobic actinomycetes: epidemiology and microbiology. Clin Microbiol Rev 7:357–417

Minnikin DE, Hutchinson IG, Caldicott AB, Goodfellow M (1980) Thin-layer chromatography of methanolysates of mycolic acid containing bacteria. J Chromatogr 188: 221–233

Mordarska H, Mordarski M, Goodfellow M (1972) Chemotaxonomic characters and classification of some nocardioform bacteria. J Gen Microbiol 71:77–86

Mordarski M, Szyba K, Pulverer G, Goodfellow M (1976) Deoxyribonucleic acid reassociation in the classification of the '*rhodochrous*' complex and allied taxa. J Gen Microbiol 94:235–245

Posada D, Crandall KA (1998) MODELTEST testing the model of DNA substitution. Bioinformatics 14:817–818

Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Saintpierre-Bonaccio D, Amir H, Pineau R, Lemriss S, Goodfellow M (2004) *Streptomyces ferralitis* sp. nov., a novel streptomycete isolated from a New-Caledonian ultramafic soil. Int J Syst Evol Microbiol 54:2061–2065

Shirling EB, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol 16:313–340

Staneck JL, Roberts GD (1974) Simplified approach to the identification of aerobic actinomycetes by thin-layer chromatography. Appl Microbiol 28:226–231

Swofford DL (1998) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts

Taha A (1983) A serological survey of antibodies of *Streptomyces somaliensis* and *Actinomadura madurae* in Sudan using enzyme-linked immunosorbent assay (ELISA). Trans R Soc Trop Med Hyg 77:49–50

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal × windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl Acids Res 25:4876–4882

Trujillo ME, Goodfellow M (2003) Numerical phenetic classification of clinically significant aerobic sporoactinomycetes and related organisms. Antonie van Leeuwenhoek 84: 39–68

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Trüper HG (1987) Report of the *ad hoc* committee on reconciliation of approaches to bacterial systematics. Int J Syst Evol Microbiol 37: 463–464

Williams ST, Goodfellow M, Alderson G, Wellington EMH, Sneath PHA, Sackin MJ (1983) Numerical classification of *Streptomyces* and related genera. J Gen Microbiol 129:1743–1813

Williams ST, Goodfellow M, Alderson G (1989) Genus *Streptomyces* Waksman and Henrici 1943, 339^{AL}. In: Williams ST, Sharpe ME, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 4. Williams and Wilkins, Baltimore, pp 2452–2492

Xu C, Wang L, Cui Q, Huang Y, Liu Z, Zhang G, Goodfellow M (2006) Neutrotolerant acidophilic *Streptomyces* species isolated from acidic soils in China: *Streptomyces guanduensis* sp. nov., *Streptomyces paucisporeus* sp. nov., *Streptomyces rubidus* sp. nov. and *Streptomyces yanglinensis* sp. nov. Int J Syst Evol Microbiol 56: 1109–1115