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Classification of thermophilic streptomycetes, including the description of *Streptomyces thermoalcalitolerans* sp. nov.

Bongcheol Kim,[1,2] Nevzat Sahin,[2]† David E. Minnikin,[3] Jolanta Zakrzewska-Czerwinska,[4] Marian Mordarski[4] and Michael Goodfellow[1,2]

Author for correspondence: Michael Goodfellow. Tel: +44 191 222 7706. Fax: +44 191 222 5228. e-mail: m.goodfellow@ncl.ac.uk

[1,2,3] Departments of Agricultural and Environmental Science[1], Microbiology[2] and Chemistry[3], University of Newcastle, Newcastle upon Tyne NE1 7RU, UK

[4] Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Czerska 12, 53-114 Wroclaw, Poland

A polyphasic taxonomic study was undertaken to clarify relationships within and between representative thermophilic alkalitolerant streptomycetes isolated from soil and appropriate marker strains. The resultant data, notably those from DNA–DNA relatedness studies, support the taxonomic integrity of the validly described species *Streptomyces thermodiastaticus*, *Streptomyces thermoviolaceus* and *Streptomyces thermovulgaris*. However, the genotypic and phenotypic data clearly show that *Streptomyces thermonitrificans* Desai and Dhala 1967 and *S. thermovulgaris* (Henssen 1957) Goodfellow *et al.* 1987 represent a single species. On the basis of priority, *S. thermonitrificans* is a later subjective synonym of *S. thermovulgaris*. Similarly, 10 out of the 11 representative thermophilic alkalitolerant isolates had a combination of properties consistent with their classification as *S. thermovulgaris*. The remaining thermophilic alkalitolerant isolate, *Streptomyces* strain TA56, merited species status. The name *Streptomyces thermoalcalitolerans* sp. nov. is proposed for this strain. A neutrophilic thermophilic isolate, *Streptomyces* strain NAR85, was identified as *S. thermodiastaticus*.

Keywords: classification, thermophilic streptomycetes, *Streptomyces thermoalcalitolerans* sp. nov.

INTRODUCTION

Systematic studies on the genus *Streptomyces* are complex due to the remarkable taxonomic variation encompassed by this taxon and the absence of recognized minimal standards for the circumscription of new species (Manfio *et al.*, 1995; Hain *et al.*, 1997). Nevertheless, it is now clear that established and putatively novel streptomycete species need to be based on a judicious selection of genotypic and phenotypic features (Chun *et al.*, 1997; Hatano *et al.*, 1997; Labeda *et al.*, 1997). Molecular systematic data are proving to be especially useful in determining the boundaries and internal taxonomic structure of the genus (Labeda, 1993, 1996; Kim *et al.*, 1996, 1998; Labeda *et al.*, 1997).

Thermophilic streptomycetes grow between 25 and 55 °C. These organisms, which grow quite well at 50 °C, have been sharply distinguished from mesophilic streptomycetes in numerical phenetic surveys (Goodfellow *et al.*, 1987; O'Donnell *et al.*, 1993); they also fall into several distinct evolutionary clades based on 16S rDNA sequence data (Kim *et al.*, 1996, 1998). It is, therefore, apparent that thermophilic streptomycetes form a diverse group and should not be recognized as a single sub-group within the genus *Streptomyces* as was proposed by Craveri & Pagani (1962).

Streptomyces macrosporus Goodfellow *et al.* 1987, *Streptomyces megasporus* (Krassilnikov *et al.* 1968) Agre 1983, *Streptomyces thermoautotrophicus* Gadkari *et al.* 1990, *Streptomyces thermocarboxydovorans* Kim *et al.* 1998, *Streptomyces thermocarboxydus* Kim *et al.* 1998, *Streptomyces thermodiastaticus* (Bergey *et al.* 1923) Waksman 1953, *Streptomyces thermolineatus* Goodfellow *et al.* 1987, *Streptomyces thermonitrificans* Desai and Dhala 1967, *Streptomyces thermoviolaceus* (Henssen 1957) emend. Goodfellow *et al.*, 1987 and *Streptomyces thermovulgaris* (Henssen 1957) emend.

†**Present address:** OMÜ Egitim Fakültesi, Biyoloji Bölümü, 55200 Atakum-Samsun, Turkey.

Table 1. Test strains

ATCC, American Type Culture Collection, Rockville, MD, USA; CUB, Culture Collection of the University of Bradford, Bradford, UK; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; ISP, International *Streptomyces* Project; NCIM, National Collection of Industrial Microorganisms, Poona, India.

Strain number	Species or subspecies	Strain history
Marker strains		
DSM 40443^T	*S. thermoviolaceus* subsp. *thermoviolaceus*	ISP 5443^T ← ATCC 19283^T ← A. Henssen; $R_{77}{}^T$, mixed fresh horse and swine manure
DSM 40444^T	*S. thermovulgaris*	ISP 5444^T ← ATCC 19284^T ← A. Henssen; $R_{10}{}^T$, cow manure
DSM 40573^T	*S. thermodiastaticus*	ISP 5573^T ← T. Cross, CUB 687 ← J. R. Denison
DSM 40579^T	*S. thermonitrificans*	ISP 5579^T ← NCIM 2007^T ← A. J. Desai & S. A. Dhala; soil, Bombay, India
DSM 41392^T	*S. thermoviolaceus* subsp. *apingens*	ATCC 19994^T ← A. Henssen, $R_{89}{}^T$
ISP 5236^T	*S. griseus*	H. J. Kutzner, Technische Hochschule, Darmstadt, Germany, DSM 40236^T
Thermophilic alkalitolerant isolates*		
A1853	*Streptomyces* sp.	J. Lacey, Rothamsted Experimental Station, Harpenden, England, UK; barley grain
A1956	*Streptomyces* sp.	J. Lacey Rothamsted Experimental Station, Harpenden, England, UK; barley grain
NT218	*Streptomyces* sp.	N. Sahin; scrubland, Merida, Venezuela
TA12, TA26, TA56, TA61, TA123, TA179, TA265	*Streptomyces* sp.	N. Sahin; garden soil, Yogyakarta, Indonesia
Thermophilic neutrophilic isolate*		
NAR85	*Streptomyces* sp.	A. T. Bull, Department of Biosciences, University of Canterbury, Kent, UK; lime soil

* Strains included in the chemosystematic and 16S rDNA sequencing studies.

Goodfellow *et al*., 1987 are validly described species which contain thermophilic streptomycetes. Additional thermophilic streptomycetes have been assigned to taxa which are not cited in the Approved Lists of Bacterial Names, notably '*Streptomyces thermoflavus*' (Kudrina and Maximova 1963) Pridham 1970, '*Streptomyces thermophilus*' (Gilbert 1904) Waksman and Henrici 1948 (syn. *Streptomyces rectus* Henssen 1957) and the illegitimately described '*Streptomyces thermotolerans*'. Several investigators have called for comparative taxonomic studies to clarify the finer taxonomic relationships between *S. thermodiastaticus*, *S. thermonitrificans*, *S. thermoviolaceus* and *S. thermovulgaris* (Goodfellow *et al*., 1987; Kim *et al*., 1996).

Sahin (1995) isolated large numbers of thermophilic streptomycetes from soil by incubating starch casein agar plates supplemented with cycloheximide and rifampicin, and adjusted to pH 10·5, at 55 °C for 5 d. In a comprehensive numerical phenetic survey of these organisms, representative isolates, which grew between pH 6 and 11·5, formed a distinct aggregate taxon. Marker strains of *S. thermovulgaris*, including the type strain, formed a distinct and homogeneous taxospecies within this aggregate taxon. Additional marker strains, such as the type strains of *S. macrosporus*, *S. megasporus*, *S. thermodiastaticus*, *S. thermolineatus* and *S. thermoviolaceus* were assigned to other aggregate taxa.

The primary aims of the present investigation were to determine relationships between representative thermophilic alkalitolerant isolates and appropriate marker strains using molecular systematic and microbiological techniques and to clarify relationships between members of established taxa, notably between *S. thermodiastaticus*, *S. thermonitrificans*, *S. thermoviolaceus* and *S. thermovulgaris*.

METHODS

Organisms and culture conditions. The strains (Table 1), which were maintained on inorganic salt-starch agar (ISP medium 4, Difco; Shirling & Gottlieb, 1966) at 45 °C and as glycerol suspensions (20 %, v/v) at −20 °C, were cultivated at 45 °C, unless otherwise stated; they all grow well at this

Table 2. Strains used and their nucleotide sequence accession numbers

Species or subspecies	Strain*	Source†	Accession no.
S. abikoensis		DSM 40831^T	X53168
S. acidiscabies		ATCC 49003^T	D63865
S. albus	ISP 5313^T	DSM 40313^T	X53163
S. ambofaciens	ISP 5053^T	ATCC 23877^T	M27245
S. bikiniensis	ISP 5581^T	DSM 40581^T	X79851
S. bluensis	ISP 5564^T		X79324
S. bottropensis	ISP 5262^T	ATCC 25435^T	D63868
S. brasiliensis		DSM 43159^T	X53162
S. caelestis	ISP 5084^T	NRRL 2418^T	X80824
S. cinnamoneus subsp. *cinnamoneus*	ISP 5005^T	DPDU 0093^T	X53171
S. diastaticus subsp. *diastaticus*	ISP 5496^T	DSM 40496^T	X53161
S. diastatochromogenes	ISP 5449^T	ATCC 12309^T	D63867
'*S. espinosus*'		NRRL 5729	X80826
S. eurythermus	ISP 5014^T	ATCC 14975^T	D63870
S. galbus	ISP 5089^T	DSM 40089^T	X79852
S. glaucescens		DSM 40716	X79322
S. griseocarneus	ISP 5004^T	DSM 40004^T	X99943
S. griseus	ISP 5236^T	KCTC 9080^T	X61478
S. lincolnensis	ISP 5355^T	NRRL 2936^T	X79854
S. macrosporus		DSM 41449^T	Z68099
S. mashuense	ISP 5221^T	DSM 40221^T	X79323
S. megasporus		DSM 41476^T	Z68100
S. mobaraensis	ISP 5587	DSM 40587	X53167
S. neyagawaensis	ISP 5588^T	ATCC 27449^T	D63869
S. olivoreticuli subsp. *cellulophilus*		DPDU 0278^T	X53166
S. pseudogriseolus		NRRL 3985	X80827
S. purpureus		DSM 43460^T	X53170
S. rimosus	R6-554		X62884
S. roseoverticillatus		DPDU 0819	X53164
S. salmonis		DPDU 0098^T	X53169
S. sampsonii	ISP 5394^T	ATCC 25495^T	D63871
S. scabiei		ATCC 49173^T	D63862
S. seoulensis		IMSNU 21266^T	Z71365
S. setonii		ATCC 25497^T	D63872
S. subrutilus	ISP 5445^T	DSM 40445^T	X80825
S. tendae	ISP 5101^T	ATCC 19812^T	D63873
S. thermocarboxydovorans	AT52^T	DSM 44296^T	U94489
S. thermocarboxydus	AT37^T	DSM 44293^T	U94490
S. thermodiastaticus	ISP 5573^T	DSM 40573^T	Z68101
S. thermolineatus		DSM 41451^T	Z68097
S. thermonitrificans	ISP 5579^T	DSM 40579^T	Z68098
S. thermoviolaceus subsp. *apingens*		DSM 41392^T	Z68095
S. thermoviolaceus subsp. *thermoviolaceus*	ISP 5443^T	DSM 40443^T	Z68096
S. thermovulgaris	ISP 5444^T	DSM 40444^T	Z68094
S. (*coelicolor*) *violaceoruber*	Strain A3(2)	DSM 41007	X60514
S. virginiae	ISP 5094^T	IFO 3729^T	D85119
Streptomyces strain	TA56	DSM 41741^T	AJ000284
Streptomyces strain	NAR85	DSM 41740	AJ001434

* ISP, International *Streptomyces* Project.

† ATCC, American Type Culture Collection, Rockville, MD, USA; DPDU, Istituto di Difesa delle Plante, Universita degli Studi di Udine, Udine, Italy; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; IFO, Institute of Fermentation, Osaka, Japan; IMSNU, Institute of Molecular Microbiology, Seoul National University, Seoul, Republic of Korea; KCTC, Korean Collection of Type Cultures, Korean Research Institute of Bioscience and Biotechnology, Taejeon, Republic of Korea; NRRL, Northern Regional Research Laboratory, Agricultural Research Service, US Department of Agriculture, Peoria, IL, USA.

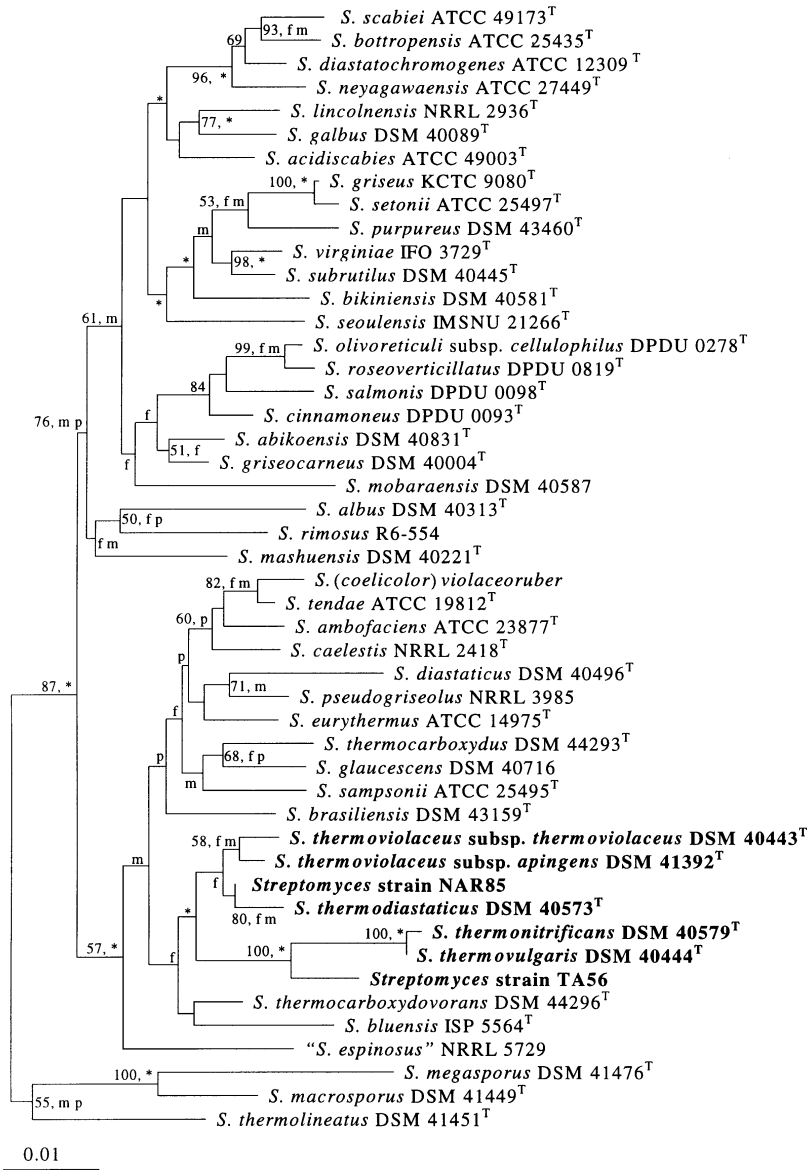

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on partial 16S rDNA sequences (< 1242 nucleotides) of 48 streptomycetes. f, m and p indicate branches that were also found with the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) algorithms, respectively; the asterisks indicate branches that were recovered with all four methods. The numbers at the nodes indicate the level (%) of bootstrap support based on a neighbour-joining analysis of 500 resampled data sets; only the values that were over 50% are given. The scale bar indicates 0·01 substitutions per nucleotide position.

temperature, which has been used in corresponding studies (Goodfellow *et al.*, 1987; Kim *et al.*, 1996, 1998). Biomass for the chemical and molecular systematic analyses was obtained by growing strains in shake flasks (approx. 150 r.p.m.) for 3 d in tryptic soy broth and harvesting by centrifugation. The cells used for the chemical studies were washed in distilled water and freeze-dried; those required for the molecular systematic investigations were washed in NaCl/EDTA buffer (0·1 M EDTA pH 8·0, 0·1 M NaCl) and stored at −20 °C until needed.

Morphology and pigmentation. The strains were examined for aerial spore mass colour and spore chain morphology following incubation on inorganic salt-starch agar for 5 d. Soluble pigment production was detected on glucose asparagine agar (ISP medium 5, Difco; Shirling & Gottlieb, 1966), and the production of melanin pigments on peptone yeast extract iron agar (ISP medium 6, Difco; Shirling & Gottlieb, 1966) and tyrosine agar (ISP medium 7, Difco; Shirling & Gottlieb, 1966). In all cases plates were incubated for 7 d. Spore chain morphology and spore surface ornamentation of *Streptomyces* strains NAR85 and TA56 were examined by scanning electron microscopy, as described previously (O'Donnell *et al.*, 1993).

Degradation and growth tests. The degradation and growth tests (Table 4) were carried out on the strains using the media and methods described by Williams *et al.* (1983). Inoculated plates were incubated for 7 d apart from some of the temperature tests. Growth at 10, 15 and 20 °C was examined after 15 d; the remaining growth tests were read after 7 d.

Chemotaxonomic analyses. The isomeric form of diaminopimelic acid of 11 of the strains (Table 1) was determined by TLC of whole-organism hydrolysates on cellulose acetate sheets as described by Staneck & Roberts (1974). Menaquinones were extracted from dried biomass of *S. thermonitrificans* DSM 40579T, *S. thermovulgaris* DSM 40444T and *Streptomyces* strain TA56, and purified using the preparative TLC procedure of Minnikin *et al.* (1984). The purified menaquinones were separated by HPLC using a Pharmacia LKB instrument fitted with a Spherisorb ODS

Table 3. Mean levels of DNA relatedness (%) found amongst representative thermophilic streptomycetes using the nitrocellulose filter method

The pairwise DNA relatedness values shown were obtained by averaging measurements of two sets of hybridizations. In each hybridization, one of a pair of DNA preparations was labelled.

Strain	Labelled strains:						
	DSM 40444^T	DSM 40579^T	TA56	DSM 40573^T	NAR85	DSM 41392^T	DSM 40443^T
S. thermovulgaris DSM 40444^T	100						
S. thermonitrificans DSM 40579^T	91	100					
Streptomyces strain TA56	62	50	100				
S. thermodiastaticus DSM 40573^T	11	12	17	100			
Streptomyces strain NAR85	8	ND	ND	97	100		
S. thermoviolaceus subsp. *apingens* DSM 41392^T	12	ND	ND	46	48	100	
S. thermoviolaceus subsp. *thermoviolaceus* DSM 40443^T	10	13	17	58	55	95	100
S. griseus ISP 5236^T*	2	2	1	8	9	6	6

ND, Not determined.

* Mesophilic *Streptomyces* control strain.

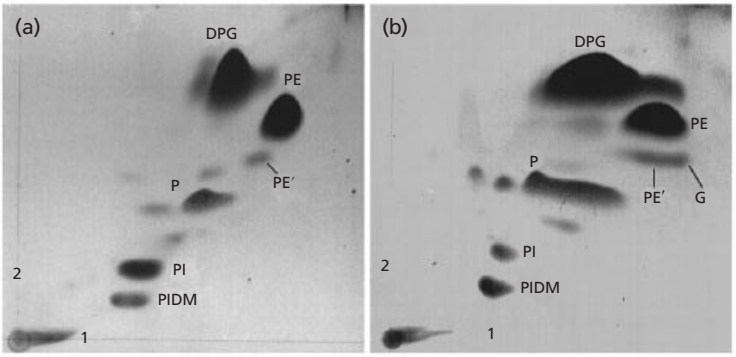

Fig. 2. Two-dimensional TLC of polar lipids of (a) *S. thermovulgaris* DSM 40444^T and (b) *Streptomyces* strain TA56. DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PE′, phosphatidylethanolamine derivative; PI, phosphatidylinositol; PIDM, phosphatidylinositol dimannoside; G, unidentified glycolipid; P, unidentified phospholipid. Numbers indicate the order of development.

column (5 μm particle size, 250 × 4·6 mm i.d.; Jones Chromatography). Polar lipids extracted from these strains were examined by two-dimensional TLC and identified using published procedures (Minnikin *et al.*, 1984).

DNA base composition. The base composition of the genomic DNA isolated from *S. thermonitrificans* DSM 40579^T, *S. thermovulgaris* DSM 40444^T and *Streptomyces* strain TA56 was determined by using the reverse-phase HPLC method described by Tamaoka (1994) and the HPLC conditions outlined by Gerke *et al.* (1984). The analyses were performed on a Supelcosil LC-18S column (Supelco) with 5 μm particle size and a column dimension of 15 cm × 4·6 mm i.d. Molar G + C ratios were calculated using the methods described by Mesbah *et al.* (1989).

Sequencing of genes encoding 16S rRNA. Extraction of genomic DNA and PCR amplification of 16S rDNA from 11 of the strains (Table 1) were carried out as described previously (Chun & Goodfellow, 1995). The amplified fragments were purified by gel electrophoresis and sequenced directly by using a *Taq* DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems) and previously described oligonucleotide primers (Chun & Goodfellow, 1995). Sequencing gel electrophoresis was carried out and the nucleotide sequences automatically obtained by using an Applied Biosystems DNA sequencer (model 373A) and software provided by the manufacturer.

Phylogenetic analysis. The 16S rDNA sequences were aligned manually with available streptomycete nucleotide sequences (Table 2) retrieved from the Ribosomal Database Project (Maidak *et al.*, 1997) and EMBL/GenBank database by using the AL16S program (Chun, 1995).

Evolutionary trees were inferred with four tree-making algorithms, namely, the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) algorithms. Evolutionary distance matrices for the least-squares and neighbour-joining methods were generated as described by Jukes & Cantor

(1969). Phylogenetic trees based on each of the algorithms were generated by using the PHYLIP package (Felsenstein, 1993). The resultant unrooted tree topologies were evaluated in light of a bootstrap analysis (Felsenstein, 1985) of the neighbour-joining method based on 500 resamplings using the SEQBOOT and CONSENSE programs in the PHYLIP package (Felsenstein, 1993).

DNA–DNA relatedness studies. Purified DNA was prepared from all of the strains following the procedure described by Mordarski *et al.* (1976). The quality of the DNA preparations was checked by taking spectrophotometric readings and by agarose gel electrophoresis of the samples (Sambrook *et al.*, 1989). DNA relatedness values between strains (Table 3) were determined in duplicated experiments using the nitrocellulose membrane filter technique (Mordarski *et al.*, 1976; Stackebrandt *et al.*, 1981). High-molecular-mass DNA was denatured by using the alkaline extraction method and immobilized single-stranded DNA was prepared by allowing denatured DNA solutions to filter by gravity or low vacuum through nitrocellulose membrane filters (0·2 μm pore size, Sartorius). Filter discs (5 mm) loaded with about 7·5 μg DNA were punched out from the nitrocellulose membranes.

DNA preparations (Table 3) were labelled with deoxy[1′,2′,5′-^{3}H]CTP using a nick translation kit (Amersham). The filter disc preparations were preincubated in 1 × Denhardt's solution at 60 °C for 1–2 h. Overnight hybridization was carried out in 200 μl 3 × SSC solution (Sambrook *et al.*, 1989) containing 35 % (w/v) deionized formamide and approximately 50000 c.p.m. of labelled DNA at 60 °C. The concentrations of SSC and the hybridization temperature were designed to achieve optimal hybridization conditions, that is, 25 °C below the melting temperature assuming that the mean DNA base composition of *Streptomyces* strains is 70 mol % G+C. After hybridization, the nitrocellulose filters were washed in 3 × SSC and thoroughly dried. The amount of bound probe DNA was estimated by scintillation counting and relatedness values expressed as the percentage of probe bound (mean of duplicated hybridizations) relative to the homologous reaction.

Ribotyping. Purified high-molecular-mass DNA (approx. 2–3 μg) was digested with *Bam*HI, *Sal*I and *Pvu*II (Boehringer Mannheim) using 10 units of enzyme per 1 μg of DNA in a 25 μl volume reaction at 37 °C overnight. The resultant DNA fragments were separated in 20 cm long agarose gels (1 %, w/v; 5 mm thick) at 40 V for 20 h at room temperature in 1 × TBE (Tris borate/EDTA; Sambrook *et al.*, 1989) buffer. The gels were stained for up to 30 min with ethidium bromide (0·5 μg ml^{-1}), photographed under UV light and transferred to a Hybond-N^{+} nylon membrane (Amersham) by using a standard Southern blotting procedure (Sambrook *et al.*, 1989), but omitting the depurination step. High-molecular-mass fragments (> 2 kb) were UV-nicked for 5 min on a UV transilluminator prior to Southern transfer.

A 7·2 kb DNA fragment containing the 16S, 23S and 5S portions of the rRNA operon of *Streptomyces* (*coelicolor*) *violaceoruber* DSM 41007 (Zakrzewska-Czerwinska, 1989) was cloned into the *Sal*I site of the pUC18 plasmid, propagated in *Escherichia coli* JM109 (Promega) and used as the probe. Digoxigenin (DIG) random primer labelling of the rDNA fragment was performed by using a commercial kit according to the manufacturer's instructions (Boehringer Mannheim, 1995).

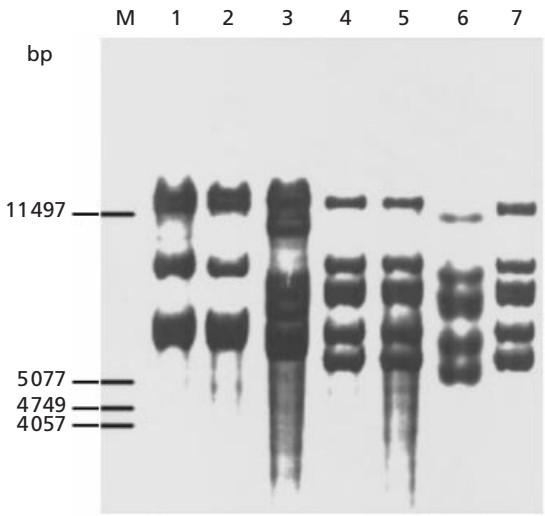

Fig. 3. Ribotyping patterns generated from *Bam*HI genomic DNA digests hybridized with the digoxigenin-labelled rDNA probe. Lanes: 1, *S. thermovulgaris* DSM 40444^{T}; 2, *S. thermonitrificans* DSM 40579^{T}; 3, *Streptomyces* strain TA56; 4, *S. thermodiastaticus* DSM 40573^{T}; 5, *Streptomyces* strain NAR85; 6, *S. thermoviolaceus* subsp. *thermoviolaceus* DSM 40443^{T}; 7, *S. thermoviolaceus* subsp. *apingens* DSM 41392^{T}; M, lambda DNA digested with *Pst*I as size marker.

RESULTS AND DISCUSSION

Numerical phenetic surveys designed to unravel the complicated taxonomic structure of the genus *Streptomyces* have yielded conflicting results with respect to members of some of the validly described taxa which contain thermophilic streptomycetes (Williams *et al.*, 1983; Goodfellow *et al.*, 1987; Kämpfer *et al.*, 1991). In particular, the taxonomic standing of *S. thermonitrificans* Desai and Dhala 1967 is not clear as the type strain of this species has been reported to share genotypic (Ochi, 1995; Kim *et al.*, 1996) and phenotypic (Goodfellow *et al.*, 1987) properties in common with both *S. thermoviolaceus* (Henssen 1957) emend. Goodfellow *et al.* 1987 and *S. thermovulgaris* (Henssen 1957) emend. Goodfellow *et al.* 1987. Similarly, *S. thermodiastaticus* (Bergey *et al.* 1923) Waksman 1953 has many phenotypic characters in common with *S. thermoviolaceus* (Henssen 1957) emend. Goodfellow *et al.* 1987, including the ability to form spores with small hemispherical warts in spiral chains (Vobis & Henssen, 1983; Goodfellow *et al.*, 1987). The ultrastructure of the hemispherical warts of *S. thermoviolaceus* was examined by Vobis & Henssen (1983) who recommended that this type of spore ornamentation be designated 'tuberculate'. The close relationship between *S. thermodiastaticus* and *S. thermoviolaceus* is also evident from 16S rDNA sequencing studies (Fig. 1). DNA–DNA homology studies have been successfully used to resolve the finer taxonomic relationships between such closely related organisms, as it is generally agreed that genomic species should encompass strains which show approximately 70 % or

Table 4. Phenotypic properties of the test strains

+, Positive, or more than 90% of strains positive in the case of the *S. thermocarboxydovorans* and *S. thermovulgaris* strains; −, negative, or more 90% of strains negative in the case of the *S. thermocarboxydovorans* and *S. thermovulgaris* strains. 1, *Streptomyces* strain TA56; 2, *S. thermocarboxydovorans*; 3, *S. thermodiastaticus* DSM 40573^T; 4, *Streptomyces* strain NAR85; 5, *S. thermoviolaceus* subsp. *thermoviolaceus* DSM 40443^T; 6, *S. thermoviolaceus* subsp. *apingens* DSM 41392^T; 7, *S. thermovulgaris*; 8, *S. thermonitrificans* DSM 40579^T.

Strains…	1	2*	3*	4	5	6*	7*	8*
Aerial spore mass colour	Grey	Grey	Grey	Grey	Grey	Grey	Grey	Grey
Pigmentation of substrate mycelium†	Not distinctive	Not distinctive	Yellowish brown	Yellowish brown	Yellow/purple‡	Yellow	Not distinctive	Not distinctive
Spore chain§	SP	RF	SP	SP	SP	SP	SP	SP
Spore surface	Warty	Smooth	Tuberculate	Tuberculate	Tuberculate	Tuberculate	Smooth	Smooth
Melanin production	−	−	−	−	−	−	−	−
Nitrate reduction	+	+	−	−	−	−	+	+
Degradation of:								
Adenine	−	+	+	+	+	+	v	+
Casein	+	ND	+	+	+	ND	+	+
DNA	+	+	−	−	+	ND	+	+
Elastin	−	+	+	+	+	+	+	+
Gelatin	+	−	+	+	+	+	+	+
Guanine	−	−	−	−	−	−	−	−
Hypoxanthine	−	+	−	−	−	+	−	−
Starch	+	+	+	+	+	+	+	+
Testosterone	+	ND	+	+	−	−	v	−
L-Tyrosine	+	+	+	+	+	+	+	+
Xanthine	−	+	−	−	−	−	−	−
Xylan	+	+	−	−	−	−	−	−
Growth on sole carbon sources:								
L-Arabinose	+	−	−	−	−	+	−	−
Fructose	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
meso-Inositol	+	ND	+	−	+	+	+	+
Mannitol	+	ND	+	+	+	+	+	+
Raffinose	−	ND	+	+	−	−	−	−
Rhamnose	+	ND	−	+	−	−	−	−
Sucrose	+	+	−	−	−	+	+	+
Xylose	+	+	+	+	+	+	+	+
Growth at:								
10 °C	−	−	−	−	−	−	−	−
15 °C	−	−	−	−	−	−	−	−
20 °C	−	+	−	−	+	+	−	−
25 °C	+	+	+	+	+	+	+	+
30 °C	+	+	+	+	+	+	+	+
37 °C	+	+	+	+	+	+	+	+
45 °C	+	+	+	+	+	+	+	+
50 °C	+	+	+	+	+	+	+	+
55 °C	+	+	+	+	+	+	+	+
60 °C	−	−	−	−	−	−	−	−
pH 6	+	+	+	+	+	+	+	+
pH 10	+	+	−	−	−	−	+	+

ND, Not determined; v, variable.

* Data taken from previous studies (Shirling & Gottlieb, 1969, 1972; Williams *et al*., 1983; Goodfellow *et al*., 1987; Sahin, 1995; Kim *et al*., 1998).

† Observed on oatmeal agar.

‡ Substrate mycelium is initially yellow but becomes purple after 5 d due to the formation of a diffusible purple pigment.

§ RF, *rectiflexibiles*; SP, *spirales*.

more DNA–DNA relatedness under suitable experimental conditions (Wayne *et al*., 1987; Stackebrandt & Goebel, 1994; Goodfellow *et al*., 1997).

The DNA homology data show that *S. thermonitrificans* DSM 40579^T and *S. thermovulgaris* DSM 40444^T belong to a single genomic species which is readily distinguished from a corresponding taxon which encompasses *S. thermoviolaceus* subsp. *apingens* and *S. thermoviolaceus* subsp. *thermoviolaceus* (Table 3). The type strains of *S. thermonitrificans* and *S. thermovulgaris* share almost identical 16S rDNA sequences (Kim *et al*., 1996), have similar ribosomal AT-L30 proteins (Ochi, 1995), have diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol dimannosides and unidentified phospholipids as major polar lipids (Fig. 2a), have octahydrogenated menaquinones with nine isoprene units as the predominant isoprenologue, and G+C-rich DNA (70 and 72 mol%, respectively). The identical ribotype patterns shown by these strains also serve to distinguish them from *S. thermodiastaticus*

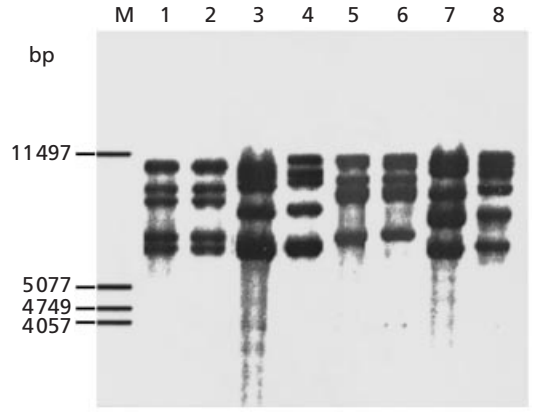

Fig. 4. Ribotyping patterns generated from *Sal*I (lanes 1–4) and *Pvu*II (lanes 5–8) genomic DNA digests hybridized with the digoxigenin-labelled rDNA probe. Lanes: 1 and 5, *S. thermodiastaticus* DSM 40573^T; 2 and 6, *Streptomyces* strain NAR85; 3 and 7, *S. thermoviolaceus* subsp. *thermoviolaceus* DSM 40443^T; 4 and 8, *S. thermoviolaceus* subsp. *apingens* DSM 41392^T; M, lambda DNA digested with *Pst*I as size marker.

and *S. thermoviolaceus* (Fig. 3) and underpins the results of an early investigation where *S. thermonitrificans* DSM 40579^T and *S. thermovulgaris* DSM 40444^T were found to produce similar randomly amplified polymorphic DNA profiles, albeit ones which were markedly different from those generated by *S. thermodiastaticus* DSM 40573^T and *S. thermoviolaceus* DSM 40443^T (Kim *et al.*, 1998). It is clear from both the present and earlier results that *S. thermonitrificans* DSM 40579^T and *S. thermovulgaris* DSM 40444^T are members of the same species. It is therefore proposed that *S. thermonitrificans* Desai and Dhala 1967 be recognized as a subjective synonym of *S. thermovulgaris* (Henssen 1957) emend. Goodfellow *et al.* 1987. This proposal supersedes an earlier one where it was proposed that *S. thermonitrificans* be accepted as a subjective synonym of *S. thermoviolaceus* (Goodfellow *et al.*, 1987). It is also clear that the thermophilic alkalitolerant *Streptomyces* (strains A1853, A1956, NT218, TA12, TA26, TA61, TA123, TA179 and TA265) are *bona fide* members of *S. thermovulgaris* as they share DNA relatedness values of over 90% with labelled DNA preparations from *S. thermovulgaris* strains DSM 40444^T and DSM 40579.

16S rDNA sequence data show that *S. thermodiastaticus* DSM 40573^T is most closely related to *S. thermocarboxydovorans* DSM 44296^T (99·0% nucleotide sequence similarity), *S. thermoviolaceus* subsp. *apingens* DSM 41392^T (99·1%), *S. thermoviolaceus* subsp. *thermoviolaceus* DSM 40443^T (99·4%) and *S. thermovulgaris* DSM 40444^T (97·7%) (Kim *et al.*, 1996, 1998). However, it is clear from the DNA relatedness data that the type strains of *S. thermodiastaticus*, *S. thermoviolaceus* and *S. thermovulgaris* belong to different genomic species (Table 3). Members of these taxa can also be distinguished from one another and from related taxa, including *S. thermocarboxydovorans*, using a combination of phenotypic properties (Table 4). *S. thermodiastaticus* DSM 40573^T was also distinguished from the type strains of the two subspecies of *S. thermoviolaceus* when genomic digests prepared using *Pvu*II and *Sal*I restriction endonucleases were probed with the 7·2 kb DNA fragment from *S.* (*coelicolor*) *violaceoruber* DSM 41007 (Fig. 4). However, all three of these strains gave similar banding patterns in the corresponding experiments with *Bam*HI genomic digests, though the profile for the *S. thermoviolaceus* subsp. *thermoviolaceus* strain showed bands with slightly lower molecular masses than those of the *S. thermoviolaceus* subsp. *apingens* strain; these differences may be due to deletions in the DNA near the rRNA operons (Fig. 3).

The inclusion of *S. thermodiastaticus* DSM 40573^T in the *Streptomyces halstedii* (Williams *et al.*, 1983) and *Streptomyces rochei* (Kämpfer *et al.*, 1991) numerical phenetic clusters can be attributed to the poor growth of this strain at the incubation temperatures used (25 and 28 °C, respectively), and to test and sampling error (Sneath & Johnson, 1972). Similar factors probably explain the assignment of *S. thermoviolaceus* DSM 40443^T to the *Streptomyces aurantiacus* (Williams *et al.*, 1983) and *Streptomyces graminofaciens* (Kämpfer *et al.*, 1991) clusters. It can be concluded from the present study that *S. thermodiastaticus* (Bergey *et al.* 1923) Waksman 1953 and *S. thermoviolaceus* (Henssen 1957) emend. Goodfellow *et al.* 1987 continue to merit recognition as validly described species.

All of the thermophilic alkalitolerant isolates (strains A1853, A1956, NT218, TA12, TA26, TA56, TA61, TA123, TA179 and TA265) share a number of phenotypic properties which are consistent with their classification in the genus *Streptomyces* (Williams *et al.*, 1989; Manfio *et al.*, 1995). They all formed an extensively branched substrate mycelium, aerial hyphae which differentiated into long chains of spores, and gave whole-organism hydrolysates that were rich in LL-diaminopimelic acid. It is evident from the DNA homology studies that all but one of the thermophilic alkalitolerant representatives of the numerical phenetic clusters recognized by Sahin (1995), namely, *Streptomyces* strain TA56, showed 90% or more DNA relatedness with reference DNA prepared from *S. thermovulgaris* DSM 40444^T (Table 3). These organisms also formed a grey aerial spore mass, lacked distinct substrate mycelium pigments, formed spores in spiral chains and were melanin-negative; all of these properties are typical of *bona fide* members of the taxon *S. thermovulgaris* (Goodfellow *et al.*, 1987; Sahin, 1995). In addition, all of these strains gave the same ribotype pattern as *S. thermovulgaris* DSM 40444^T when *Bam*HI genomic digests were hybridized with the rDNA probe (Fig. 3).

Streptomyces strain TA56 formed a distinct single-membered cluster (Sahin, 1995), albeit one related to phena shown in this investigation to accommodate *S. thermovulgaris* strains. The 16S rDNA (Fig. 1) and

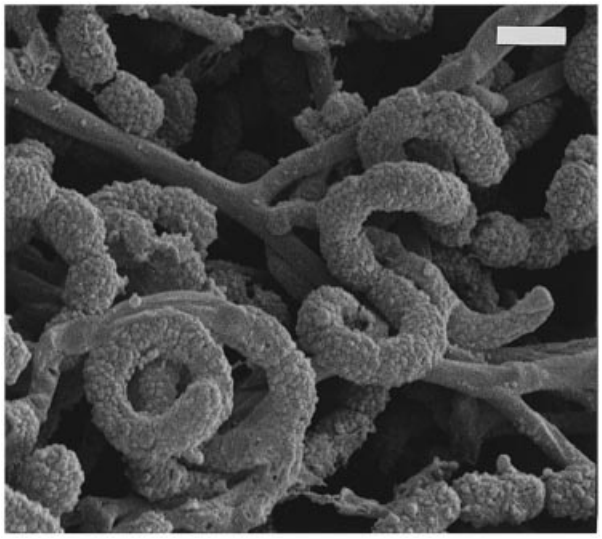

Fig. 5. Scanning electron micrograph showing coiled spore chains with warty surface of *Streptomyces* strain TA56. The organism was grown on inorganic salt-starch agar (ISP medium 4) at 45 °C for 5 d. Bar, 1 μm.

DNA relatedness (Table 3) data are in good agreement with the results of the numerical taxonomic study. *Streptomyces* strain TA56 showed its closest 16S rDNA sequence similarity with *S. thermovulgaris* DSM 40444^T (98·9 % or 1271 out of 1285 nucleotides shared) and a corresponding high DNA relatedness value (Table 3), though one well below the 70 % cut-off point used to assign strains to single genomic species (Wayne *et al.*, 1987; Goodfellow *et al.*, 1997). A number of phenotypic features can also be weighted to differentiate *Streptomyces* strain TA56 from *S. thermovulgaris* strains (Table 4), not least the ability of the former to form warty-surfaced (Fig. 5) as opposed to smooth spores. The two strains can also be distinguished by their polar lipid patterns as *Streptomyces* strain TA56 produced an unidentified glycolipid (α-naphthol- and periodate/Schiff-positive) which co-migrated with a phosphatidylethanolamine derivative spot (Fig. 2a, b). *Streptomyces* strain TA56 and *S. thermovulgaris* DSM 40444^T gave different ribotype patterns when high-molecular-mass DNA from these organisms was digested with *Bam*HI and treated with the rDNA probe prepared from *S.* (*coelicolor*) *violaceoruber* DSM 41007 (Fig. 3). It is interesting that strain TA56 has octahydrogenated menaquinone with nine isoprene units as the predominant isoprenologue like *S. thermovulgaris* DSM 40444^T and DSM 40579.

It is clear from both the genotypic and phenotypic data that *Streptomyces* strain TA56 is related to, but distinct from, *S. thermovulgaris*. It can also be differentiated from *S. thermoautotrophicus* for, unlike the latter, it is not an obligate chemolithoautotroph and does not grow at 65 °C (Gadkari *et al.*, 1990). Accordingly, the new species *Streptomyces thermoalcalitolerans* is proposed for *Streptomyces* strain TA56.

Description of *Streptomyces thermoalcalitolerans* sp. nov.

Streptomyces thermoalcalitolerans (ther.mo.al.ca.li.-to′le.rans. Gr. n. *therme* heat; N.L. n. *alcali* from arabic *al* end; *galiy* soda ash; L. pres. part. *tolerans* tolerating, enduring; M.L. part. adj. *thermoalcalitolerans* thermophilic alkali-tolerating).

The description is based on data taken from this and an earlier study (Sahin, 1995). Aerobic, Gram-positive, thermophilic actinomycete with extensively branched substrate and aerial hyphae. Spiral chains of warty surfaced spores are borne on aerial hyphae. The aerial spore mass is grey; neither distinctive substrate mycelium colours nor diffusible pigments are formed. Melanin pigments are not produced on peptone iron agar. Casein, DNA, gelatin, starch, testosterone, L-tyrosine and xylan are degraded, but not adenine, arbutin, elastin, guanine, hypoxanthine or xanthine. Adonitol, L-arabinose, arabitol, D-cellobiose, D-fructose, D-galactose, D-glucose, *meso*-inositol, α-lactose, D-mannitol, D-mannose, D-melezitose hydrate, melibiose, α-L-rhamnose, D-ribose, D-sorbitol, sucrose, D-trehalose, D-turanose, xylitol and D-xylose are used as sole carbon sources for energy and growth, but D-raffinose is not. Growth occurs between 25 and 55 °C, from pH 6·0 to 11·5, and in the presence of ampicillin (8 μg ml^{-1}), bacitracin (16 μg ml^{-1}), oleandomycin phosphate (16 μg ml^{-1}), penicillin G (15 international units), rifampicin (16 μg ml^{-1}), streptomycin sulphate (4 μg ml^{-1}), tetracycline hydrochloride (16 μg ml^{-1}) and tunicamycin (10 μg ml^{-1}). In contrast, growth is inhibited in the presence of gentamicin sulphate (8 μg ml^{-1}), lincomycin hydrochloride (32 μg ml^{-1}), neomycin sulphate (8 μg ml^{-1}), novobiocin (4 μg ml^{-1}), oleandomycin phosphate (32 μg ml^{-1}), polymyxin B phosphate (32 μg ml^{-1}), rifampicin (32 μg ml^{-1}), streptomycin sulphate (16 μg ml^{-1}), tetracycline hydrochloride (32 μg ml^{-1}), tobramycin sulphate (32 μg ml^{-1}) and vancomycin hydrochloride (16 μg ml^{-1}). The DNA base composition of the organism is 73 mol % G+C. Isolated from tropical garden soil collected by M. Goodfellow in 1991 from Yogyakarta, Central Java, Indonesia. The type strain, TA56 (= DSM 41741) is deposited in the DSMZ, Braunschweig, Germany.

Neutrophilic isolate

The only neutrophilic thermophilic isolate included in this study, *Streptomyces* strain NAR85, formed a single-membered cluster in the numerical phenetic survey of Sahin (1995). This taxon formed an aggregate group together with *S. thermodiastaticus* DSM 40573^T and *S. thermoviolaceus* DSM 40443^T. The close association with *S. thermodiastaticus* was underpinned by the results of the DNA homology studies as strain NAR85 showed 97 % DNA relatedness with labelled DNA prepared from *S. thermodiastaticus* DSM 40573^T. This assignment is also supported by phenotypic (Table 4), ribotyping (Figs 3, 4) and 16S rDNA

sequence data. The sequence data show that strain NAR85 is closely related to *S. thermodiastaticus* DSM 40573^T (99·5 % nucleotide sequence similarity) and *S. thermoviolaceus* DSM 40443^T (99·4 %). It is clear from both the genotypic and phenotypic data that strain NAR85 belongs to the species *S. thermodiastaticus*. *Streptomyces* strain NAR85 has been deposited in the DSMZ, Braunschweig, Germany, under the accession number DSM 41740.

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REFERENCES

Agre, N. S. (1983). Genera *Streptomyces*, *Streptoverticillium* and *Chainia*. In *A Guide for the Determination of Actinomycetes*, pp. 52–71. Edited by T. F. Gause, T. P. Preobrazhenskaya, M. A. Sveshnikova, L. P. Terekhova & T. S. Maksimova. Moscow: Nauka (in Russian).

Bergey, D. H., Harrison, F. C., Breed, R. S., Hammer, B. W. & Huntoon, F. M. (1923). *Bergey's Manual of Determinative Bacteriology*, 1st edn. Baltimore: Williams & Wilkins.

Chun, J. (1995). *Computer-assisted classification and identification of actinomycetes*. PhD thesis, University of Newcastle upon Tyne.

Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 240–245.

Chun, J., Youn, H.-D., Yim, Y.-I., Lee, H., Kim, M. Y., Hah, Y. C. & Kang, S.-O. (1997). *Streptomyces seoulensis* sp. nov. *Int J Syst Bacteriol* **47**, 492–498.

Craveri, R. & Pagani, H. (1962). Thermophilic micro-organisms among actinomycetes in the soil. *Ann Microbiol* **12**, 115–130.

Desai, A. J. & Dhala, S. A. (1967). *Streptomyces thermonitrificans* sp. n., a thermophilic streptomycete. *Antonie Leeuwenhoek* **33**, 137–144.

Felsenstein, J. (1981). Evolutionary tree from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

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

Felsenstein, J. (1993). PHYLIP (Phylogenetic Inference Package), version 3.5c. Department of Genetics, University of Washington, Seattle, USA.

Fitch, W. M. & Margoliash, E. (1967). Construction of phylogenetic trees: a method based on mutation distances as estimated from cytochrome *c* sequences is of general applicability. *Science* **155**, 279–284.

Gadkari, D., Schricker, K., Acker, G., Kroppenstedt, R. M. & Meyer, O. (1990). *Streptomyces thermoautotrophicus* sp. nov., a thermophilic CO- and H_2-oxidizing obligate chemolithotroph. *Appl Environ Microbiol* **56**, 3727–3734.

Gerke, C. W., McCune, R. A., Gama-Sosa, M. A., Ehrlich, M. & Kuo, K. C. (1984). Quantitative reversed-phase high-performance liquid chromatography of major and modified nucleosides in DNA. *J Chromatogr* **301**, 199–219.

Gilbert, R. (1904). Über *Actinomyces thermophilus* und andere Actinomyceten. *Z Hyg Infektionskr* **47**, 383–406.

Goodfellow, M., Lacey, J. & Todd, C. (1987). Numerical classification of thermophilic streptomycetes. *J Gen Microbiol* **133**, 3135–3149.

Goodfellow, M., Manfio, G. P. & Chun, J. (1997). Towards a practical species concept for cultivatable bacteria. In *Species: the Units of Biodiversity*, pp. 25–59. Edited by M. F. Claridge, H. A. Dawah & M. R. Wilson. London: Chapman & Hall.

Hain, T., Ward-Rainey, N., Kroppenstedt, R. M., Stackebrandt, E. & Rainey, F. A. (1997). Discrimination of *Streptomyces albidoflavus* strains based on the size and number of 16S-23S ribosomal DNA intergenic spacers. *Int J Syst Bacteriol* **47**, 202–206.

Hatano, K., Nishii, T. & Mordarska, H. (1997). *Streptomyces spitzbergensis* Wieczorek *et al*. 1993 is a later subjective synonym of *Streptomyces baldacii* (Farina and Locci 1966) Witt and Stackebrandt 1991. *Int J Syst Bacteriol* **47**, 573–574.

Henssen, A. (1957). Über die Bedeutung der thermophilen Mikroorganismen für der Zersetzung des Stallmistes. *Arch Mikrobiol* **27**, 63–81.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Kämpfer, P., Kroppenstedt, R. M. & Dott, W. (1991). A numerical classification of the genera *Streptomyces* and *Streptoverticillium* using miniaturized physiological tests. *J Gen Microbiol* **137**, 1831–1891.

Kim, D., Chun, J., Sahin, N., Hah, Y.-C. & Goodfellow, M. (1996). Analysis of thermophilic clades within the genus *Streptomyces* by 16S ribosomal DNA sequence comparisons. *Int J Syst Bacteriol* **46**, 581–587.

Kim, S. B., 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.

Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.

Krassilnikov, N. A., Agre, N. S., Dorokhova, L. A. & Sokolov, A. A. (1968). Three new species of thermophilic actinomycetes. *Mikrobiologiya* **37**, 75–83 (in Russian).

Kudrina, E. S. & Maximova, T. S. (1963). Some species of thermophilic *Actinomyces* from soils of China and their antibiotic properties. *Mikrobiologiya* **32**, 623–631 (in Russian).

Labeda, D. P. (1993). DNA relatedness among strains of the *Streptomyces lavendulae* phenotypic cluster group. *Int J Syst Bacteriol* **43**, 822–825.

Labeda, D. P. (1996). DNA relatedness among verticil-forming *Streptomyces* species (formerly *Streptoverticillium* species). *Int J Syst Bacteriol* **46**, 699–703.

Labeda, D. P., Lechevalier, M. P. & Testa, R. T. (1997). *Streptomyces stramineus* sp. nov., a new species of the verticillate streptomycetes. *Int J Syst Bacteriol* **47**, 747–753.

Maidak, B. L., Olsen, G. J., Larsen, N., Overbeek, R., McCaughey, M. J. & Woese, C. R. (1997). The Ribosomal Database Project (RDP). *Nucleic Acids Res* **25**, 109–111.

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

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise

measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athayle, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

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.

Ochi, K. (1995). A taxonomic study of the genus *Streptomyces* by analysis of ribosomal protein AT-L30. *Int J Syst Bacteriol* **45**, 507–514.

O'Donnell, A. G., Falconer, C., Goodfellow, M., Ward, A. C. & Williams, E. (1993). Biosystematics and diversity amongst novel carboxydotrophic actinomycetes. *Antonie Leeuwenhoek* **64**, 325–340.

Pridham, T. G. (1970). New names and new combinations in the order *Actinomycetales* Buchanan 1917. *Tech Bull ARS USDA* **1424**, 1–55.

Sahin, N. (1995). *Selective isolation, characterisation and classification of novel thermotolerant streptomycetes*. PhD thesis, University of Newcastle upon Tyne.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

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

Shirling, E. B. & Gottlieb, D. (1969). Cooperative description of type cultures of *Streptomyces*. IV. Species descriptions from the second, third and fourth studies. *Int J Syst Bacteriol* **19**, 391–512.

Shirling, E. B. & Gottlieb, D. (1972). Cooperative description of type strains of *Streptomyces*. V. Additional descriptions. *Int J Syst Bacteriol* **22**, 265–394.

Sneath, P. H. A. & Johnson, R. (1972). The influence on numerical taxonomic similarities of error in microbiological tests. *J Gen Microbiol* **72**, 377–392.

Stackebrandt, E. & Goebel, B. M. (1994). A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Stackebrandt, E., Wunner-Füssl, B., Fowler, V. J. & Schleifer, K.-H. (1981). Deoxyribonucleic acid homologies and ribosomal ribonucleic acid similarities among sporeforming members of the order *Actinomycetales*. *Int J Syst Bacteriol* **31**, 420–431.

Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.

Tamaoka, J. (1994). Determination of DNA base composition. In *Chemical Methods in Prokaryotic Systematics*, pp. 463–470. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.

Vobis, G. & Henssen, A. (1983). Ultrastructure of tuberculate spores in *Streptomyces thermoviolaceus*. *Arch Microbiol* **134**, 295–298.

Waksman, S. A. (1953). Description of species of *Streptomyces*. In *Guide to the Classification and Identification of Actinomycetes and their Antibiotics*, p. 102. Edited by S. A. Waksman & H. A. Lechevalier. Baltimore: Williams & Wilkins.

Waksman, S. A. & Henrici, A. T. (1948). Family III *Streptomycetaceae* Waksman and Henrici. In *Bergey's Manual of Systematic Bacteriology*, 6th edn, pp. 929–980. Edited by R. S. Breed, E. G. D. Murray & P. Hitchens. Baltimore: Williams & Wilkins.

Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983). Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129**, 1743–1813.

Williams, S. T., Goodfellow, M. & Alderson, G. (1989). Genus *Streptomyces* Waksman and Henrici 1943, 339^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2452–2492. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

Zakrzewska-Czerwinska, J. (1989). *Organization of the ribosomal RNA genes* in *Streptomyces strains*. PhD thesis, Ludwik Hirszfeld Institute, Wroclaw, Poland.