Numerical Classification of Thermophilic Streptomycetes

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Fifty thermophilic streptomycetes from diverse habitats were compared through 135 unit characters with 201 representative mesophilic streptomycetes from an earlier numerical phenetic study. Data were examined using the simple matching (S_{SM}), Jaccard (S_J) and pattern (D_P) coefficients, and clustering was achieved using the unweighted pair group method with arithmetic averages (UPGMA) technique. In all three analyses, the thermophilic streptomycetes formed an aggregate taxon composed of three major (seven to nineteen strains), five minor (two to three strains) and two single-member clusters. Cluster composition was not affected by the statistics used. The numerical phenetic data showed that thermophilic streptomycetes form several distinct centres of variation, four of which correspond to previously described species; a further taxon was also considered to merit species status. It is proposed that *Streptomyces thermolineatus* sp. nov. be recognized and the name *Streptomyces macrosporus* Krassilnikov *et al.* 1968 be revived. Emended descriptions are given for *Streptomyces megasporus* (Krassilnikov *et al.*, 1968) Agre 1983, *Streptomyces thermoviolaceus* Henssen 1957 and *Streptomyces thermovulgaris* Henssen 1957.

INTRODUCTION

Thermophilic streptomycetes have received little attention from taxonomists despite their potential importance in microbial technology (Kutzner, 1981). *Streptomyces thermodiastaticus* (Bergey *et al.*, 1923) Waksman 1953, *Streptomyces thermonitrificans* Desai & Dhala 1967, *Streptomyces thermoviolaceus* Henssen 1957 and *Streptomyces thermovulgaris* Henssen 1957 are cited in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) and *Streptomyces megasporus* (Krassilnikov *et al.*, 1968) and *Streptomyces glaucosporus* (Krassilnikov *et al.*, 1968), have recently been revived and validated (Agre, 1983, 1986) but '*Streptomyces macrosporus*' (Krassilnikov *et al.*, 1968), '*Streptomyces thermoflavus*' (Kudrina & Maksimova, 1963) Pridham 1970, '*Streptomyces thermofuscus*' (Waksman *et al.*, 1939) Waksman & Henrici 1948, '*Streptomyces thermophilus*' (Gilbert, 1904) Waksman & Henrici 1948 (syn. *Streptomyces rectus*; Henssen, 1957) or the illegitimately described '*Streptomyces thermotolerans*' are not listed in the Approved Lists and have not been validly published since 1 January 1980.

Thermophilic streptomycetes have growth temperature ranges between 28 °C and 55 °C, although cultures of '*S. thermofuscus*' and '*S. thermophilus*' have been reported to grow at 65 °C (Waksman *et al.*, 1939). It is a matter of some controversy whether streptomycetes that grow at or above 45 °C should be assigned to distinct taxa or whether they should be considered only as thermotolerant variants of mesophilic species. Craveri & Pagani (1962) proposed the subgenus *Thermostreptomyces* for thermophilic taxa but other workers (Corbaz *et al.*, 1963; Küster & Locci, 1963) regarded such organisms as thermotolerant rather than thermophilic. The name *Thermostreptomyces* was listed under *genera incertae sedis* in the eighth edition of *Bergey's Manual of Determinative Bacteriology* (Pridham & Tresner, 1974).

Table 1. *Description and source of thermophilic strains assigned to clusters in the* S_{SM}, *UPGMA analysis*

Strain no.	Name as received†	Source history‡
	(a) Strains assigned to cluster 1 (*Streptomyces thermovulgaris*)	
*K27	S. thermovulgaris	A. Seino, KCC S-0520; T. Hasegawa, IFO 13089; SAJ; E. B. Shirling, ISP 5444; A. Henssen, MB R_{10}
K1, K2, K5	Streptomyces sp.	C. Todd, garden compost C1, Newcastle upon Tyne
K3, K4, K8–K10, K13, K14, K16	Streptomyces sp.	C. Todd, garden compost C2, Newcastle upon Tyne
K11	Streptomyces sp.	C. Todd, mushroom compost, UK
K31	Streptomyces sp.	C. Lyons, Department of Microbiology, The University, Newcastle upon Tyne, CL4, garden compost
K34	Streptomyces sp.	J. Lacey, A372; bagasse, Jamaica
K35	Streptomyces sp.	J. Lacey, A563; bagasse, Trinidad
K40	Streptomyces sp.	J. Lacey, A902; coffee dust, Trinidad
K41	Streptomyces sp.	J. Lacey, A928; mushroom compost, UK
K42	Streptomyces sp.	J. Lacey, A956; cocoa beans, Trinidad
	(b) Strains assigned to cluster 2 (*Streptomyces thermoviolaceus*)	
*K25	'S. thermoflavus'	A. Seino, KCC S-0135 (ISP 5574); T. Cross, CUB 75; NCIB 9670; N. Okafor; rotting maize, Ado-Ekiti, Nigeria
*K26	S. thermonitrificans	A. Seino, KCC S-0841; IFO 13473; SAJ; ISP 5579; ATCC 23385; A. J. Desai, NCIMB 2007; soil, Bombay, India
K22	S. thermoviolaceus	J. Lacey, CD183; grain dust, Canada
K33	S. thermoviolaceus	J. Lacey, A74; hay, Devon, UK
K32	S. thermoviolaceus subsp. apingens	J. Lacey, A71; hay, Rothamsted, Harpenden, UK
K23	S. thermoviolaceus subsp. thermoviolaceus	J. Lacey, A221; bagasse, Trinidad
K24	S. thermoviolaceus subsp. thermoviolaceus	J. Lacey, A74; hay, Rothamsted, UK
	(c) Strains assigned to cluster 3 (*Streptomyces* sp.)	
K6, K18	Streptomyces sp.	C. Todd, garden compost C2
K12	Streptomyces sp.	C. Todd, garden compost C1
	(d) Strains assigned to cluster 4 (*Streptomyces* sp.)	
K28–K30	Streptomyces sp.	C. Lyons, CL1, CL2, CL3; garden compost
	(e) Strains assigned to cluster 5 (*Streptomyces macrosporus*)	
K19	'S. macrosporus'	J. Lacey, A1488; sewage compost, USA
*K44	'S. macrosporus'	J. Lacey, A1201; N. S. Agre, 2892
	(f) Strains assigned to cluster 6 (*Streptomyces megasporus*)	
K20	'S. macrosporus'	J. Lacey, A1489; sewage compost, USA
K38, K39	'S. macrosporus'	J. Lacey, A848, A849; air, hayfield, Rothamsted, Harpenden, UK
K43	'S. macrosporus'	J. Lacey, A1062; mushroom compost, Suffolk, UK
K46	'S. macrosporus'	J. Lacey, A1362; air, cotton mill, Lancashire, UK
K48–K50	'S. macrosporus'	J. Lacey, A1485, A1486, A1487; sewage compost, USA
*K45	S. megasporus	J. Lacey, A1202; N. S. Agre, 1869
	(g) Strains assigned to cluster 7 (*Streptomyces thermolineatus*)	
K21, K47	'S. macrosporus'	J. Lacey, A1601, A1484; sewage compost, USA
	(h) Strains assigned to cluster 8 (*Streptomyces albus*)	
K7, K15, K17	Streptomyces sp.	C. Todd, garden compost C1
	(i) Single member clusters	
K37	Streptomyces sp.	J. Lacey, A600; barley grain, Cambridge, UK
K36	Streptomyces sp.	J. Lacey, A592; bagasse, USA

Williams *et al.* (1983) made an extensive numerical phenetic survey of the type strains of *Streptomyces* species in which '*S. thermoflavus*' ISP 5574, *S. thermonitrificans* ISP 5579 and *S. thermovulgaris* ISP 5444 were recovered in a single cluster and four other strains considered to be thermophilic (received as *S. thermodiastaticus*, '*S. thermophilus*', '*S. thermotolerans*' and *S. thermoviolaceus*) were assigned to the periphery of numerically defined groups containing mesophilic strains. Thus, *S. thermodiastaticus* ISP 5573 was recovered in subcluster 1C (*S. halstedii*), '*S. thermophilus*' ISP 5365 (*S. rectus*) in cluster 15 (*S. chromofuscus*), '*S. thermotolerans*' ISP 5227 in cluster 18 (*S. cyaneus*) and *S. thermoviolaceus* ISP 5443 in cluster 45 (*S. aurantiacus*).

The primary aim of the present study was to clarify the taxonomy of thermophilic streptomycetes by comparing phenetic data from representative strains with corresponding results obtained on marker mesophilic strains examined by Williams *et al.* (1983). The two data sets were compared using standard numerical taxonomic techniques.

METHODS

Isolation of thermophilic streptomycetes. Two samples of garden compost, and one of mushroom compost, were incubated at 55 °C for 7 d. Preincubated samples (1 g each) were shaken separately in 10 ml of quarter-strength Ringer's Solution (Oxoid, BR52) on a Griffin flask shaker (Griffin & George) at speed setting 8 for 30 min. Samples (0·2 ml) of 10^{-2} to 10^{5} serial, logarithmic dilutions were spread over the surface of starch-casein nitrate agar plates (Küster & Williams, 1964) supplemented with actidione (50 µg ml^{-1}; Sigma) and rifampicin (0·5 µg ml^{-1}; Sigma) (five plates per dilution) and incubated at 55 °C for 7 d. Streptomycete counts were expressed as numbers of colony-forming units (c.f.u.) (g dry wt of sample)$^{-1}$; dry weights were obtained at 105 °C. Eighteen randomly selected isolates with the colony appearance and morphology of streptomycetes, from isolation plates with 30–100 colonies, were subcultured on starch-casein nitrate agar plates, incubated at 45 °C for 5 d and checked for purity by microscopic examination of Gram-stained smears. Other isolates were obtained from samples of mouldy hay, sugar-cane bagasse, cereal grain, coffee dust, cocoa beans, mushroom compost, and from a sewage compost using an Andersen sampler/wind tunnel method (Gregory & Lacey, 1963; Lacey & Dutkiewicz, 1976) or from air over pastures and in a cotton mill using an Andersen sampler (Lacey, 1975; Lacey & Lacey, 1987).

Fifty thermophilic strains comprising 45 isolates together with the type strains of five *Streptomyces* species were examined (Table 1). Strains were maintained on modified Bennett's agar slopes (Jones, 1949) at 4 °C and as suspensions of spores or mycelial fragments in glycerol (20%, v/v) at -25 °C (Wellington & Williams, 1978).

Collection of data. Cultures were examined using all but four of the tests described by Williams *et al.* (1983); strains were not tested for xylan degradation, *Klebsiella* β-lactamase inhibitor or β-lactamase production on YPG or Beecham's FS agars. The test regimes of Williams *et al.* (1983) were followed except that, apart from measuring growth temperature requirements, all tests were incubated at 45 °C and read after 3 and 7 d. The final test readings were used to provide data for computation. Tests were repeated only when ambiguous or clearly unexpected results were obtained. The data were added to the corresponding results obtained with the streptomycetes assigned to the major and minor clusters recovered by Williams *et al.* (1983). The final data matrix contained 251 strains and 135 unit characters.

Coding of data. Nearly all of the characters existed in one of two mutually exclusive states and were scored plus (1) or minus (0). Qualitative multistate characters, such as pigmentation and spore-chain morphology, were coded as several independent characters scored plus (1) for the character state shown and minus (0) for all alternatives. Quantitative multistate characters, such as tolerance to chemical inhibitors, were scored using the additive method of Sneath & Sokal (1973).

Computation. Data were examined using the Clustan 1C program (Wishart, 1978) on an IBM 370/180 computer, using the simple matching coefficient (S_{SM}; Sokal & Michener, 1958), which includes both positive and negative similarities, the Jaccard coefficient (S_J; Sneath, 1957), which includes positive matches only, and the pattern coefficient (D_P; Wishart, 1978), which allows for differences in growth rates, periods of incubation and similar

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* Type strain.

† Binomials in inverted commas were not included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) and have not been validly published since January 1980.

‡ KCC, Culture Collection of Actinomycetes, Kaken Chemical Co. Ltd, 6-42 Jujodai 1-chome, Kita-ku, Tokyo 114, Japan; IFO, Institute for Fermentation, Yodogawa-ku, Osaka, Japan; SAJ, Society for Actinomycetes, Tokyo, Japan; ISP, International Streptomyces Project reference number; CUB, Culture Collection, University of Bradford, Yorkshire, UK; NCIB, National Collection of Industrial Bacteria, Torry Research Institute, Aberdeen.

factors that can distort similarity values (Sneath, 1968). Clustering was achieved using the unweighted pair group method with averages (UPGMA) algorithm (Sneath & Sokal, 1973).

Morphological studies. Additional morphological data on isolates were obtained using half-strength nutrient and V-8 juice agars (Corbaz *et al.*, 1963) and ISP media (Shirling & Gottlieb, 1966). Cultures were incubated at 25 and 40 °C and examined *in situ* on the agar surface using a compound microscope equipped with × 20 and × 40 objectives. The colour series of the spore mass was determined by comparison with standard colour tabs as described by Tresner & Backus (1963).

Spores, collected by touching collodion-coated copper grids onto sporulating cultures, were examined without further treatment using a Siemens Elmiskop 1A transmission electron microscope. For scanning electron microscopy, spores were mounted on stubs using double-sided adhesive tape and coated with gold before examination in a Hitachi 5450 scanning electron microscope.

RESULTS

The garden compost samples C1 and C2 contained 4·9–5·0 × 10^5 c.f.u. of thermophilic streptomycetes (g dry wt of sample)$^{-1}$ and the mushroom compost 10^3 c.f.u. (g dry wt)$^{-1}$. The thermophilic streptomycete content of other samples differed with storage conditions, most being found in mouldy hay and sugar-cane bagasse after spontaneous heating to > 50 °C, when grey thermophilic *Streptomyces* numbered up to 10^5 g^{-1} and *S. albus* up to 10^7 g^{-1}. Other species isolated included *Thermoactinomyces* species, *Faenia rectivirgula* and *Saccharomonospora viridis*.

Numerical classification

The thermophilic streptomycetes formed a distinct aggregate cluster. All the remaining strains were placed in the major and minor clusters recognized by Williams *et al.* (1983) and, with the exception of *S. albus*, formed a separate aggregate cluster. The mesophilic streptomycetes have not, therefore, been considered further.

The classification based on the S_{SM}, UPGMA analysis is described in detail as it gave the most compact clusters. The 50 test isolates were recovered in three major (seven to nineteen strains), five minor (two to three strains) and two single-member clusters at the 82% similarity (S)-level (Fig. 1). Where possible, clusters were named after the earliest described species that they contained.

Cluster 1 was the largest and was defined just below the 90% S-level. It contained the type strain of *S. thermovulgaris* and 18 other strains from different habitats (see Table 1). Cluster 1 showed a close similarity (> 80%) to cluster 2, which was also homogeneous and circumscribed just above the 90% S-level. In addition to isolates received as *S. thermoviolaceus*, cluster 2 contained type and reference strains of *S. thermonitrificans* and '*S. thermoflavus*', respectively. The third major cluster, cluster 6, was defined at the 84% S-level. It included the type strain of *S. megasporus* and eight other isolates from air and composts. The type strain of '*S. macrosporus*' formed minor cluster 5 with one other isolate from compost. The remaining clusters did not contain marker strains and were labelled *Streptomyces* sp. Clusters 3, 4 and 8 each contained three isolates from garden compost and cluster 7 contained duplicate strains from sewage compost; two other isolates were recovered as single-member clusters. The cluster 8 strains were classified as *S. albus* as they were closely associated with cluster 16 of Williams *et al.* (1983), which contained the type strain of *S. albus* and two other strains which had all been cultivated at 25 °C.

The same clusters were recovered in the S_J and D_P, UPGMA analyses. In the S_J analysis, cluster groups were defined at the 62% S-level but clusters were recovered in the same order as in the S_{SM} analysis, with no change in composition. In the D_P analysis, clusters 1 and 2 and clusters 5 and 6 were closely related and there was a loose association between clusters 4 and 8 but, apart from this, the results corresponded to those obtained in the S_{SM} UPGMA analysis.

Characterization of the clusters

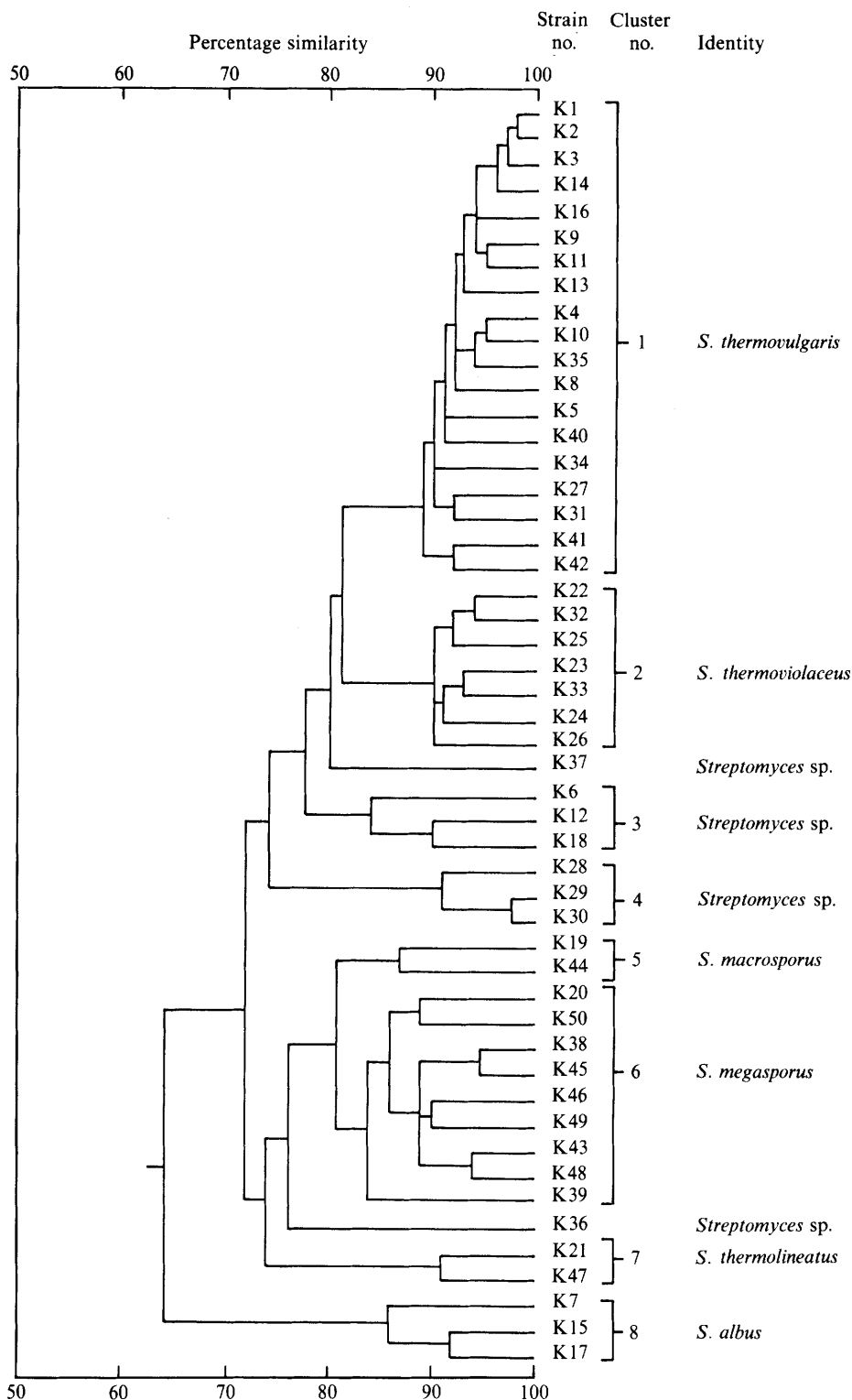Table 2 lists the properties of the different clusters and identifies characters with potential diagnostic value.

Fig. 1. Dendrogram showing the relationships between clusters recovered in the S_{SM}, UPGMA analysis.

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Table 2. *Distribution of positive characters to major, minor and single-member clusters defined at the 82% similarity level (S_{SM})*

Values are the number of strains with positive character states. Character states which are most representative of and consistent within each major cluster are marked with an asterisk.

	Major clusters			Minor clusters						
	S. thermovulgaris	*S. thermoviolaceus*	*S. megasporus*	*Streptomyces* sp.	*Streptomyces* sp.	*S. macrosporus*	*S. thermolineatus*	*S. albus*	*Streptomyces* sp.	*Streptomyces* sp.
Cluster no.	1	2	6	3	4	5	7	8	–	–
No. of strains/strain no.	19	7	9	3	3	2	2	3	K36	K37
Spore chain morphology:										
Rectiflexibiles	0	0	0	3	3	0	2	0	0	0
Retinaculiaperti	3	0	1	0	0	0	0	0	0	0
Spirales	16*	7*	8*	0	0	2	0	3	1	1
Spore surface ornamentation:										
Smooth	19*	0	0	1	2	0	2	3	0	1
Warty	0	7*	9*	0	0	2*	0	0	1	0
Spiny	0	0	0	2	1	0	0	0	0	0
Colour of aerial spore mass:										
Red	0	2	0	0	0	0	0	0	0	0
Yellow	0	0	5	1	0	0	0	0	0	0
Grey	18*	5	0	0	0	0	0	0	1	1
Green	0	0	0	0	0	2	2	0	0	0
Blue	0	0	0	2	0	0	0	0	0	0
White	1	0	4	0	3	0	0	2	0	0
Pigmentation:										
No distinctive substrate mycelial pigments	19	1	9	3	3	2	2	3	0	1
Red/orange	0	6*	0	0	0	0	0	0	1	0
Pigmentation of diffusible pigments:										
Production of diffusible pigments	0	3	1	0	0	0	0	0	0	0
Red/orange	0	2	0	0	0	0	0	0	0	0
Yellow/brown	0	0	1	0	0	0	0	0	0	0
Blue	0	1	0	0	0	0	0	0	0	0
Sensitivity of substrate pigment to pH	0	6	0	0	0	0	0	0	0	0
Sensitivity of diffusible pigment to pH	0	3	0	0	0	0	0	0	0	0
Growth of sole nitrogen source (0·1%, w/v):										
DL-α-Amino-n-butyric acid	12	7	1	2	3	1	1	2	1	0
Potassium nitrate	7	7	0	3	3	2	2	1	0	0
L-Cysteine	10	7	0	2	3	0	0	1	1	0
L-Valine	19	7	2	3	2	1	2	2	1	0
L-Threonine	19	7	0*	2	3	2	1	3	0	1
L-Serine	19	7	0*	2	3	1	2	2	1	1
L-Phenylalanine	19	7	1	3	3	2	2	2	0	0
L-Methionine	19	7	0*	2	3	0	2	3	0	1
L-Histidine	19	7	4	3	3	1	2	3	1	1
L-Arginine	19	7	0*	3	3	2	2	3	0	0
L-Hydroxyproline	0	0	1	3	3	0	2	3	1	1
Enzyme activity:										
Proteolysis on egg yolk	19	7	9	3	0	2	1	3	1	1
Lipolysis	19	7	9	3	3	2	1	3	1	1
Pectin hydrolysis	0	0	2	1	3	2	0	0	1	1
Nitrate reduction	19	1*	0*	3	1	0	1	0	0	1
Hydrogen sulphide production	18	6	9	3	0	2	0	3	1	0

Table 2—*continued*

	Major clusters			Minor clusters						
	S. thermovulgaris	*S. thermoviolaceus*	*S. megasporus*	*Streptomyces* sp.	*Streptomyces* sp.	*S. macrosporus*	*S. thermolineatus*	*S. albus*	*Streptomyces* sp.	*Streptomyces* sp.
Cluster no.	1	2	6	3	4	5	7	8	–	–
No. of strains/strain no.	19	7	9	3	3	2	2	3	K36	K37
Degradation of:										
Hippurate	0	0	2	0	0	0	0	3	0	0
Hypoxanthine	0	1	0	0	0	0	0	1	0	0
Elastin	5	7	9	2	1	1	1	1	0	0
L-Tyrosine	19	7	8	3	3	2	1	2	0	0
Adenine	0	7	0	0	0	0	0	0	0	0
DNA	19	7	9	3	0	2	0	2	1	1
RNA	19	7	9	2	0	2	0	2	1	1
Starch	19	7	9	3	0	2	2	0	0	0
Urea	1	0	0	0	0	0	0	3	0	0
Gelatin	19	7	9	3	1	2	2	0	1	1
Aesculin	9	7	9	3	0	2	0	0	0	1
Arbutin	19	7	0*	3	0	2	0	3	0	1
Resistance to antibiotics ($\mu g\ ml^{-1}$)†:										
Tobramycin (50)	19	2	9	0	2	2	0	3	1	0
Rifampicin (50)	19	7	9	2	3	1	0	3	0	1
Cephaloridine (100)	19	0*	8	3	3	2	2	3	1	1
Vancomycin (50)	12	0	0	3	0	0	0	2	0	0
Demethylchlortetracycline (500)	0	0	0	0	2	0	0	3	0	0
Oleandomycin (100)	16	0*	9	3	2	2	0	3	1	1
Lincomycin (100)	19	6	9	3	2	2	2	3	0	0
Penicillin (10 i.u.)	18	1	9	3	3	2	1	3	1	1
Growth at:										
10%	0	0	0	0	0	0	2	0	0	1
Growth in presence of (%, w/v):										
Sodium chloride (4)	19	5	5	0	0	0	0	3	1	1
Sodium chloride (7)	0	0	0	0	0	0	0	3	0	0
Sodium chloride (10)	0	0	0	0	0	0	0	2	0	0
Sodium chloride (13)	0	0	0	0	0	0	0	2	0	0
Sodium azide (0·01)	19	7	9	1	1	1	2	3	1	1
Sodium azide (0·02)	19	7	9	1	1	0	0	3	0	1
Phenyl ethanol (0·3)	19	2	9	0	0	2	0	0	1	1
Phenol (0·1)	19	7	9	2	1	2	1	1	0	1
Potassium tellurite (0·001)	19	7	9	3	1	2	1	3	0	1
Potassium tellurite (0·01)	19*	4	0	1	1	0	0	0	0	0
Thallous acetate (0·001)	17	0	4	0	0	1	0	0	0	1
Thallous acetate (0·01)	0	0	0	0	0	0	0	0	0	1
Crystal violet (0·0001)	4	5	0	1	0	0	2	1	0	1
Growth on sole carbon sources (0·1%, w/v):										
L-Arabinose	0	5	0	0	3	2	0	0	0	1
Sucrose	19	2	0	3	3	0	0	3	0	1
D-Xylose	19	7	2	3	3	2	0	3	0	1
meso-Inositol	19	7	2	0	3	2	0	2	1	1
Mannitol	19	7	0*	1	3	0	1	3	0	1
D-Fructose	19	7	0*	2	3	2	0	3	0	1
L-Rhamnose	0	0	4	3	3	2	0	0	0	1
Raffinose	0	0	0	0	0	0	0	0	1	0
D-Melezitose	17*	0	0	1	3	0	0	2	0	0

Table 2—continued

	Major clusters			Minor clusters						
	S. thermovulgaris	*S. thermoviolaceus*	*S. megasporus*	*Streptomyces* sp.	*Streptomyces* sp.	*S. macrosporus*	*S. thermolineatus*	*S. albus*	*Streptomyces* sp.	*Streptomyces* sp.
Cluster no.	1	2	6	3	4	5	7	8	–	–
No. of strains/strain no.	19	7	9	3	3	2	2	3	K36	K37
D-Mannose	19	7	5	1	3	2	0	3	0	1
D-Lactose	0	7*	1	0	3	0	0	3	1	1
Inulin	5	6	1	2	3	1	0	3	0	1
Adonitol	1	0	0	2	3	0	0	3	0	0
Salicin	0	0	0	0	0	0	0	3	0	0
Trehalose	19	7	3	3	3	2	2	3	1	1
D-Melibiose	0	1	0	0	0	0	0	0	0	1
Dextran	3	0	0	0	0	0	0	2	0	0
D-Galactose	19	7	3	1	3	0	0	3	0	1
Cellobiose	19	7	2	3	3	1	1	3	0	1
Xylitol	0	0	0	0	3	0	0	0	0	0
Sodium acetate (0·1%, w/v)	0	4	0	0	0	0	0	3	0	0
Sodium citrate (0·1%, w/v)	0	5	0	0	0	0	0	3	0	0
Sodium malonate (0·1%, w/v)	0	0	0	0	0	0	0	0	0	0
Sodium propionate (0·1%, w/v)	10	5	3	2	0	1	0	3	1	0
Sodium pyruvate (0·1%, w/v)	0	4	0	0	0	0	0	3	0	0

† Strains were tested for their ability to grow in the presence of freeze-dried filter paper discs previously soaked in antibiotic at the concentration shown (Goodfellow & Orchard, 1974)

All of the strains produced spores in an aerial spore mass, degraded Tween 80 and casein, were resistant to gentamicin (100 μg ml⁻¹), neomycin (50 μg ml⁻¹) and streptomycin (100 μg ml⁻¹), and grew at 37 °C and 45 °C and in the presence of phenyl ethanol (0·1%, v/v).

None of the strains produced verticillate spore chains, hairy or rugose ornamented spores, a violet aerial spore mass, green, blue or violet coloured substrate mycelium, green or violet diffusible pigments, or melanin pigments on either peptone yeast iron or tyrosine agars, and none showed fragmentation or spores on the substrate mycelium, produced sclerotia, exhibited antimicrobial activity against *Pseudomonas fluorescens* NCIB 9046, showed lecithinase activity on egg yolk, degraded chitin, guanine, xanthine, testosterone or allantoin, or grew either at 4 °C or at pH 4·3.

Morphology

Morphological observations are summarized in Tables 2 and 3. Strains in clusters 3 and 4 (*Streptomyces* spp.) grew insufficiently well for detailed morphological observations to be made. Organisms assigned to clusters 1 (*S. thermovulgaris*) and 2 (*S. thermoviolaceus*), and to the two single-member clusters, all had spore masses in the grey colour series, but the spore chains of the cluster 1 strains often became hygroscopic perhaps through autolysis, collapsing onto the agar and becoming dark brown to black. Strains in clusters 5 ('S. macrosporus'), 6 (*S. megasporus*) and 7 (*Streptomyces* sp.) all produced green spore masses but sometimes in cluster 6 spores were close to tab 24½ dc in the yellow colour series, perhaps when sporulation was poor. The spore masses of cluster 8 strains (*S. albus*) were white following incubation at 25 °C but became pink during incubation at 40 °C. Tight knotted spiral spore chains were characteristic of several clusters (5, 6, 8, K36, K37; Fig. 4a) while the spiral spore chains of the *S. thermoviolaceus* and *S.*

Table 3. *Summary of the morphological characteristics of thermophilic streptomycetes assigned to the different clusters*

Cluster	Spore colour series*	Spore chain morphology	Spore surface	Spore size (μm) Range	Spore size (μm) Mean
1. *S. thermovulgaris*	Grey 3 li–5 ih	Spirals <3 turns 10–30 spores	Smooth	0·8–1·5 × 0·6–1·0	0·97 × 0·79
2. *S. thermoviolaceus*	Grey d–4 ig	Spirals <2 turns <10–30 spores	Warty	0·7–1·4 × 0·5–0·9	0·95 × 0·67
5. *S. macrosporus*	Green 24 ih	Spirals <6 turns <50 spores	Wrinkled by SEM; warty by TEM	0·7–1·8 × 0·7–1·6	1·12 × 0·96
6. *S. megasporus*	Green–yellow 1½ ge–24 ih–24½ dc	Spirals <6 turns <30 spores	Wrinkled by SEM; warty by TEM	0·8–1·8 × 0·5–1·2	1·11 × 0·77
7. *S. thermolineatus*	Green 24 ih–24½ ih	Straight <30 spores	Smooth	1·0–2·1 × 0·9–1·3	1·43 × 1·06
8. *S. albus*	White–red 3 ca–5 ca	Spirals <4 turns 10–50 spores	Smooth	0·6–1·0 × 0·3–0·6	0·78 × 0·48
–. *Streptomyces* sp. K36	Grey 2 dc–5 fe	Spirals <4 turns <10–30 spores	Warty	0·8–1·8 × 0·5–1·0	1·23 × 0·73
–. *Streptomyces* sp. K37	Grey 2 fc–3 fc	Spirals 3–4 turns 10–30 spores	Smooth	0·6–1·7 × 0·6–1·0	i·05 × 0·75

* Spore mass colour series after Tresner & Backus (1963).

thermovulgaris strains were often imperfectly formed, appearing straight, hooked or looped (Fig. 2a, b). Spore chains in cluster 7 were straight (Fig. 5a).

The spores of *S. thermovulgaris* strains were smooth but those of *S. thermoviolaceus* strains were characteristically covered by small hemispherical warts 30–70 nm in diameter (Fig. 2c), as were spores of isolate K36. Spores of the 'S. macrosporus' and S. megasporus strains also resembled one another closely, appearing to have pronounced warts by transmission electron microscopy (Fig. 4b) but with irregular ridges and warts by scanning electron microscopy (Fig. 3). Spores of the remaining strains were smooth but those of the duplicate cultures in cluster 7 (*S. thermolineatus*) were unusual in shape, the ends of the spores being long, and projecting from the oval spore body (Fig. 5b). The ends retained their shape when the rest of the spore collapsed under vacuum to give a phalangiform appearance in both transmission and scanning electron microscopy (Tresner et al., 1966). Only *S. albus* isolates formed spore chains at 25 °C and these were generally similar to those at 40 °C.

DISCUSSION

Our findings extend those of Williams et al. (1983), who found that the type strains of 'S. thermoflavus', S. thermonitrificans and S. thermovulgaris, incubated at 45 °C, formed a single cluster. In our three analyses, the thermophilic streptomycetes were recovered in an aggregate cluster distinct from the aggregate clusters formed by mesophilic streptomycetes. Of especial interest, in the S_{SM} classification, were the two adjoining and closely related clusters in the thermophilic streptomycete aggregate taxon formed by S. albus isolates. Our cluster 8 contained isolates examined at 45 °C and cluster 16 of Williams et al. (1983) those, including the type strain, incubated at 25 °C. By contrast, the type strains of S. thermodiastaticus, 'S. thermophilus',
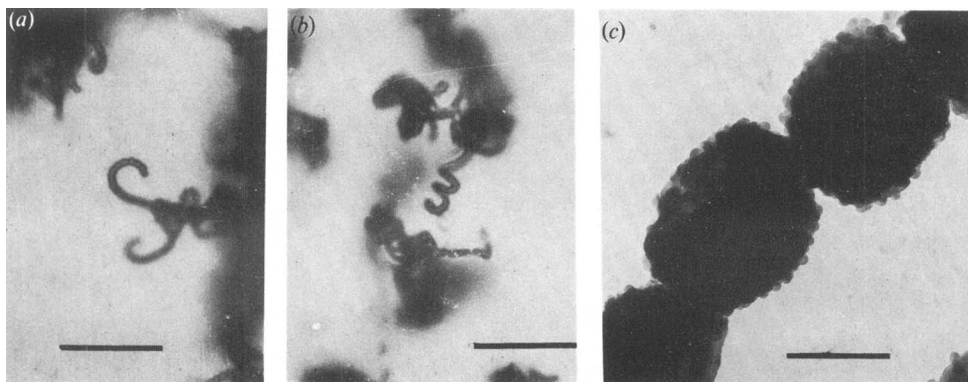
Fig. 2. (*a, b*) *S. thermoviolaceus* K23, spore chains. Bars, 20 μm. (*c*) *S. thermoviolaceus* K24, transmission electron micrograph of spores. Bar, 1 μm.
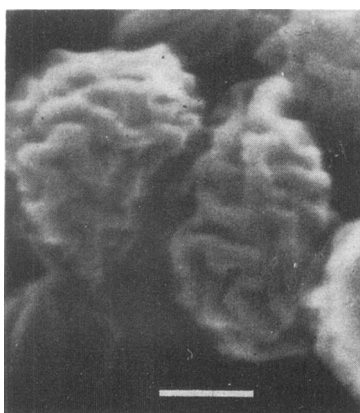

Fig. 3. *S. macrosporus* K19, scanning electron micrograph of spores. Bar, 0·5 μm.

'*S. thermotolerans*' and *S. thermoviolaceus* were recovered by Williams *et al.* (1983) at the periphery of numerically defined clusters containing mesophilic streptomycetes. This can be attributed to incubation of these strains at 25 °C, when conditions may barely have allowed growth, and to test and sampling error (Sneath & Johnson, 1972; Austin & Colwell, 1977). In the present study, *S. thermoviolaceus* marker strains were studied at 45 °C and recovered in cluster 2. The present findings strongly suggest that thermophilic streptomycetes are not merely variants of established mesophilic taxa, but further comparative work is required to determine the effect of cultivating strains at different temperatures, and especially near the limits for growth, on the stability of numerical taxonomies. However, the examination of actinobacteria at two different temperatures only marginally affected their position in the subsequent numerical classification (Goodfellow *et al.*, 1985).

The thermophilic streptomycetes contrasted with the mesophilic strains in that none produced melanin pigments, they seldom exhibited activity against allantoin, chitin, guanine, hypoxanthine, testosterone, urea and xanthine, and all failed to grow in the presence of 7% (w/v) NaCl. Also, few thermophilic strains grew at 10 °C or used compounds such as D-melibiose, raffinose, salicin and xylitol as sole carbon sources.

The numerical classification was unaffected by the coefficients used (S_{SM}, S_J, D_P) and several properties could be weighted to enable recognition of the three major clusters. It is evident that
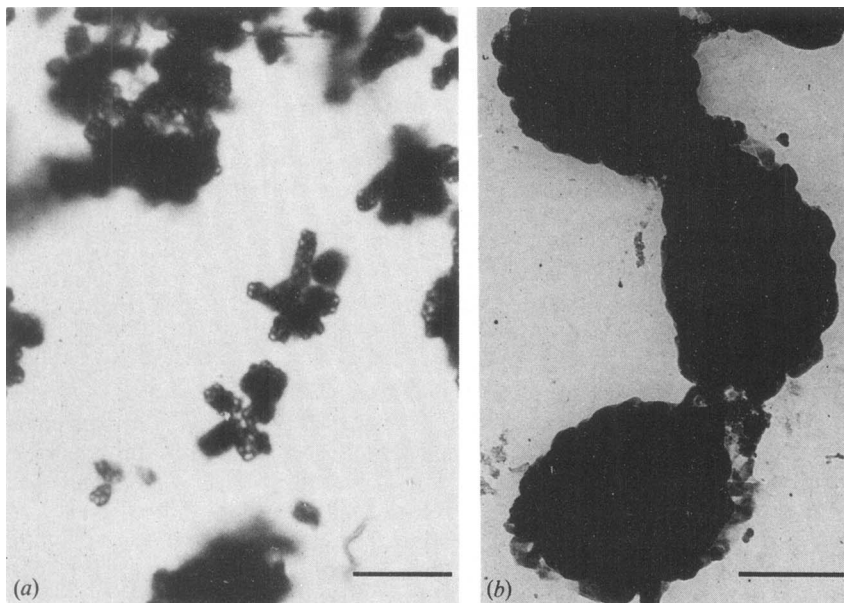
Fig. 4. (*a*) *S. megasporus* K43, spore chains. Bar, 20 μm. (*b*) *S. megasporus* K39, transmission electron micrograph of spores. Bar, 0·5 μm.
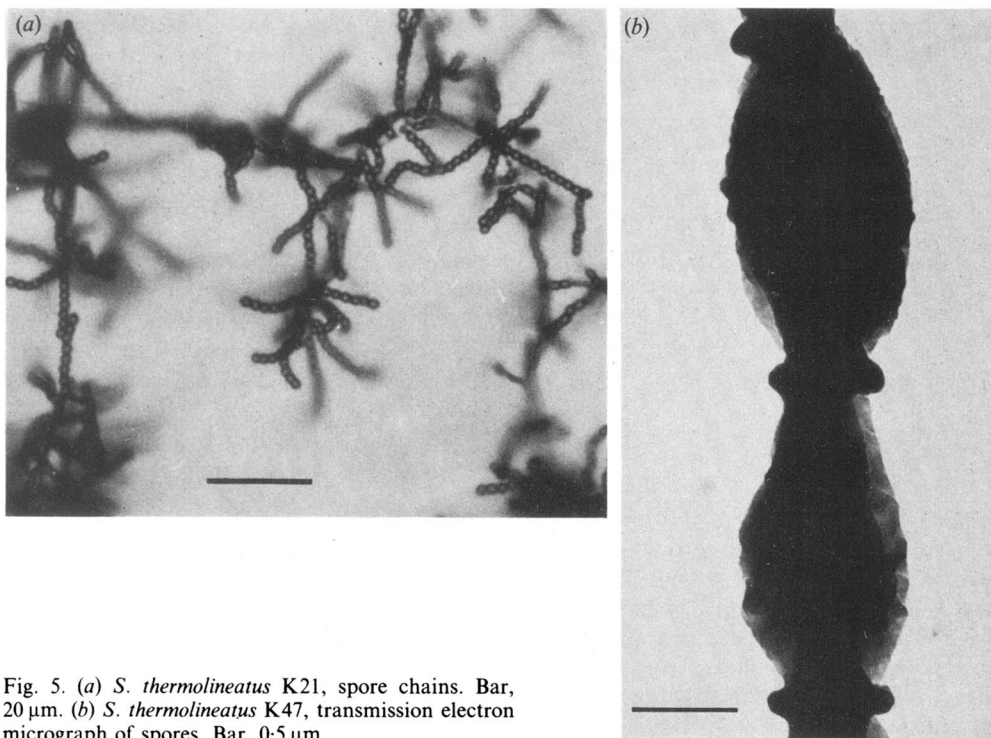

Fig. 5. (*a*) *S. thermolineatus* K21, spore chains. Bar, 20 μm. (*b*) *S. thermolineatus* K47, transmission electron micrograph of spores. Bar, 0·5 μm.

S. thermoviolaceus Henssen 1957 and *S. thermovulgaris* Henssen 1957 are good taxospecies and that *S. thermonitrificans* Desai & Dhala 1967 and '*S. thermoflavus*' (Kudrina & Maksimova, 1963) Pridham 1970 fall within the boundaries of the former. Indeed, Desai & Dhala (1967) only separated *S. thermonitrificans* and *S. thermoviolaceus* by a few biochemical and physiological properties that would now be considered insufficient for speciation (Williams *et al.*, 1983). The recovery of *S. thermonitrificans* ISP 5579 and *S. thermovulgaris* ISP 5444 in a single cluster in the study of Williams *et al.* (1983) can be attributed to the small number of thermophilic streptomycetes examined and to the fact that single marker strains are seldom good representatives of species (Wilkinson & Jones, 1977; Goodfellow *et al.*, 1982).

Further comparative studies are required to establish the taxonomic affinities of *S. thermodiastaticus* ISP 5573, which was not included in this study. This organism was classified in a cluster (Cluster 1C; *Streptomyces halstedii*; Williams *et al.*, 1983) shown to be heterogeneous on the basis of DNA : DNA pairing data (Mordarski *et al.*, 1986). *S. thermodiastaticus* (Bergey *et al.*, 1923) Waksman 1953 has many properties in common with *S. thermoviolaceus* Henssen 1957, including the ability to form spores with small hemispherical warts, in hooked and spiral chains. If synonymous with *S. thermoviolaceus* the epithet *thermodiastaticus* would have priority.

The third major phenon, cluster 6, can similarly be said to form a good taxospecies. This cluster corresponds to *S. megasporus* (Krassilnikov *et al.*, 1968) Agre 1983 as it contains the type of this species. On the same basis, minor cluster 5 corresponds to '*S. macrosporus*' Krassilnikov *et al.* 1986. Cluster 8, as shown earlier, corresponds to *S. albus* (Rossi-Doria, 1891) Waksman & Henrici 1943. Cluster 7 is a distinctive new species for which the name *Streptomyces thermolineatus* is proposed. The remaining minor phena may form nuclei of previously undescribed species, but additional isolates and further work are needed to establish this.

Description of *Streptomyces macrosporus* (*ex* Krassilnikov *et al.*, 1968, 66) nom. rev. sp. nov.

Ma.cro.spor'us. Gr. adj. *makros* long, large; Gr. n. *spora* seed; M.L. adj. *macrosporus* large-spored.

Growth at 40 °C on half-strength nutrient and V-8 juice agars good; aerial mycelium in the green colour series near 24 ih (Tresner & Backus, 1963), with white flecks, although may be thin and white on half-strength nutrient agar. At 25 °C, small colonies only, sometimes with sparse white aerial mycelium. Substrate mycelium colourless to dark brown with no distinctive pigments but often crystalline deposits in the agar. Melanoid pigments not produced on peptone iron agar. No soluble pigments.

Spores mostly in tight spirals of up to six turns and 50 spores but sometimes in short straight chains of only five spores. Spores appear warty in transmission electron micrographs but are characteristically wrinkled in scanning electron micrographs, 0·7–1·8 × 0·7–1·6 μm, sometimes broader than long, mean 1·12 × 0·96 μm (Fig. 3).

Degrades aesculin, arbutin, casein, DNA, gelatin, RNA, starch and L-tyrosine; utilizes D-fructose, *meso*-inositol, D-mannose, L-rhamnose, trehalose and D-xylose as sole carbon sources, and L-arginine, L-phenylalanine, potassium nitrate and L-threonine as sole nitrogen sources. Proteolysis and lipolysis evident on egg yolk agar; pectin hydrolysed; hydrogen sulphide produced but nitrate not reduced.

Isolated from sewage compost and soil.

Type culture: DSM 41449 = K44 = A1201 = INMI 2892.

Description of *Streptomyces megasporus* (*ex* Krassilnikov *et al.* 1968, 66) Agre 1983, VP emend.

Me.ga.spor'us. Gr. adj. *megas* big; Gr. n. *spora* seed; M.L. adj. *megasporus* big-spored.

Growth at 40 °C on half-strength nutrient and V-8 juice agars good; aerial mycelium in the green colour series, between 1½ ge and 24 ih or sometimes in the yellow series near 24½ dc. Growth at 25 °C poor, colourless and slimy in appearance with little or no aerial mycelium. Substrate mycelium colourless to olive grey on nutrient agar or dark brown on V-8 juice agar, no distinctive pigments. Yellow-brown soluble pigment sometimes produced; no melanoid pigment on peptone iron agar.

Spore chains up to 30 spores long in tight spirals of up to six turns, although frequently only one or two turns (Fig. 4a). Spores appear warty in transmission electron micrographs, and with irregular warts and ridges in scanning electron micrographs, 0·8–1·8 × 0·5–1·2 μm, mean 1·11 × 0·77 μm (Fig. 4b).

Degrades aesculin, casein, DNA, elastin, gelatin, RNA, starch and L-tyrosine; few isolates used any of the sole carbon and sole nitrogen sources tested but all caused proteolysis and lipolysis of egg yolk and produced hydrogen sulphide while none reduced nitrate to nitrite.

Isolated from air over a hayfield and in a cotton mill, from soil, mushroom compost and sewage compost.

Type culture: DSM 41450 = K45 = A1202 = Agre 1869.

Description of *Streptomyces thermolineatus* Goodfellow, Lacey & Todd sp. nov

Ther.mo.line.a′ta. Gr. f. n. *thermē* heat; L. adj. *lineatus*, of a line, rectilinear. L. f. n. *thermolineata* heat(-loving), linear (referring to spore chains).

Good growth at 40 °C on V-8 juice agar, producing abundant aerial mycelium in the green colour series, 24 ih to 24½ ih. Colony reverse yellow-brown with no distinctive pigments. No melanoid pigment produced on peptone iron agar.

Spores in straight or flexuous chains less than 30 spores long (Fig. 5a). Spores smooth but ends often prolonged, projecting from the oval spore body and retaining their shape under vacuum when the rest of the spore collapses, to give a phalangiform appearance (Fig. 5b; Tresner *et al.*, 1966). Spores measure 1·0–2·1 × 0·9–1·3 μm, mean 1·43 × 1·06 μm.

Degrades casein, gelatin and starch, uses trehalose and sometimes cellobiose and mannitol as sole carbon sources, and L-arginine, L-histidine, L-hydroxyproline, L-methionine, L-phenylalanine, potassium nitrate, L-serine and L-valine as sole nitrogen sources. Reduces nitrate to nitrite and sometimes shows lipolytic and proteolytic activity on egg yolk.

Isolated from sewage compost.

Type culture: DSM 41451 = K47 = A1484.

Description of *Streptomyces thermoviolaceus* Henssen 1957, 388[AL] emend.

Ther.mo.vi.o.la′ceus. Gr. f. n. *thermē* heat; L. adj. *violaceus* violet-coloured; M. L. adj. *thermoviolaceus* heat(-loving), violet-coloured (soluble pigment).

Synonyms: '*Streptomyces thermoflavus*' (Kudrina & Maksimova, 1963, 626) Pridham 1970, 27; *Streptomyces thermonitrificans* Desai & Dhala 1967, 626.

Grows well at 40 °C on V-8 juice, yeast/malt and glycerol/asparagine agars, producing plane or convolute colonies with aerial mycelium, but poorly on half-strength nutrient agar to give thin colourless colonies that often lack aerial mycelium. At 25 °C, growth is very restricted and colourless substrate mycelium only is produced. May grow also at 55 °C, producing thin colonies with little or no aerial mycelium. The sporulating aerial mycelium is various shades in the grey colour series (Tresner & Backus, 1963), with white flecks. Substrate mycelium colourless, yellow or dark brown, sometimes modified by yellow or purple soluble pigment, the latter pH-sensitive, turning red with acid. Melanoid pigments not produced on peptone iron agar.

Spore chains in open hooks or short spirals of up to two turns, usually fewer than 10 spores but occasionally up to 30 (Fig. 2a, b). Spores oval, 0·7–1·4 × 0·5–0·9 μm, mean 0·95 × 0·67 μm, characteristically with small hemispherical warts, about 30–70 nm diameter, covering the surface (Fig. 2c) but sometimes appearing smooth.

Degrades adenine, aesculin, arbutin, casein, DNA, elastin, gelatin, RNA, starch and L-tyrosine; uses cellobiose, D-fructose, D-galactose, *meso*-inositol, D-lactose, mannitol, mannose, trehalose and xylose as sole carbon sources, and DL-α-amino-n-butyric acid, L-arginine, L-cysteine, L-histidine, L-methionine, L-phenylalanine, potassium nitrate, L-serine, L-threonine and L-valine as sole nitrogen sources. Proteolysis and lipolysis evident on egg yolk, hydrogen sulphide produced, but nitrate not reduced.

Isolated from soil dung and from moulding hay, cereal grains and sugar-cane bagasse.

Type culture: ATCC 19283.

Description of *Streptomyces thermovulgaris* Henssen 1957, 391[AL] emend.

Ther.mo.vul.ga′ris. Gr. f. n. *thermē* heat; L. adj. *vulgaris* common; M.L. adj. *thermovulgaris* heat(-loving), common.

Growth moderate to good at 40 °C on half-strength nutrient and V-8 juice agars and often also on yeast/malt and glycerol/asparagine agars although sometimes with sparse aerial mycelium only. At 25 °C little or no growth, without aerial mycelium. Sporulating aerial mycelium in the grey colour series corresponding to tabs 3 li to 3 ig or 5 ih unless aerial mycelium is sparse, when only d to dc (Tresner & Backus, 1963). Aerial mycelium often becoming hygroscopic, collapsing onto the agar to form dark brown to black areas. Colony reverse colourless to dark brown with no distinctive pigments. Melanoid pigments not produced on peptone iron agar. No soluble pigments.

Spores produced in straight, hooked and spiral chains with up to three turns, less than 10–50 spores long. Spores globose to oval, smooth or slightly roughened, 0.8–1.5 × 0.6–1.0 μm, mean 0.97 × 0.79 μm.

Degrades arbutin, casein, DNA, gelatin, RNA, starch and L-tyrosine; utilises cellobiose, D-fructose, D-galactose, *meso*-inositol, mannitol, D-mannose, sucrose, trehalose and D-xylose as sole carbon sources, and L-arginine, L-histidine, L-methionine, L-phenylalanine, L-serine, L-threonine and L-valine as sole nitrogen sources. Proteolysis and lipolysis evident on egg yolk; hydrogen sulphide produced, and nitrate reduced.

Isolated from fresh and rotted faeces of cows, sheep and horses, garden and mushroom composts, sugar-cane bagasse, cocoa beans and coffee dust.

Type culture: ATCC 19284 = K27.

The present study provides further evidence that thermophilic streptomycetes are widely distributed in nature, particularly in compost heaps and decaying vegetable matter where temperatures of at least 65 °C occur (Henssen, 1957; Lacey, 1973). The improved classification of these organisms should facilitate ecological studies in substrates such as fodders and composts.

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