Saccharopolyspora gregorii and *Saccharopolyspora hordei*: Two New Actinomycete Species from Fodder

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Spore-forming actinomycetes from fodder were compared with marker strains of the genera *Amycolata*, *Amycolatopsis*, *Faenia*, *Saccharopolyspora* and *Saccharothrix*. Data from 120 unit characters were examined using the pattern and simple matching coefficients, and clustering was achieved using the unweighted pair group method with arithmetic averages algorithm. Most of the fodder isolates were recovered in two major clusters which were equated with taxospecies. Representatives of the numerically circumscribed species contained *meso*-diaminopimelic acid, arabinose and galactose, were rich in *iso*- and *anteiso*- fatty acids, exhibited tetrahydrogenated menaquinones with nine isoprene units as the predominant isoprenologue and gave similar polar lipid patterns. The chemical, morphological and numerical phenetic data indicated that the organisms belong to the genus *Saccharopolyspora*. It is proposed that the two novel taxa be given species status within the genus *Saccharopolyspora* as *Saccharopolyspora gregorii* sp. nov. and *Saccharopolyspora hordei* sp. nov.

INTRODUCTION

Actinomycetes are common in fodder (Lacey, 1988), where they grow rapidly on heating and produce degradative enzymes that enable utilization of the substrate. Members of some taxa cause extrinsic allergic alveolitis (hypersensitivity pneumonitis) resulting from inhalation of large numbers of spores released when mouldy substrates are disturbed (Lacey, 1981). Species such as *Faenia rectivirgula*, *Saccharomonospora viridis*, *Streptomyces albidoflavus* and *Streptomyces albus* are found in a wide range of substrates but other taxa, notably *Nocardiopsis dassonvillei*, *Saccharopolyspora hirsuta*, *Streptomyces thermoviolaceus* and *Thermomonospora chromogena*, seem to be favoured by particular substrates (Lacey, 1988). Fodder isolates whose taxonomic status is uncertain include strains initially assigned to the genus *Actinomadura* (Lacey *et al.*, 1978) but subsequently shown to form three numerically circumscribed taxa, clusters A, B and C (Athalye, 1981), that had little in common with phena corresponding to established species of *Actinomadura* (Goodfellow *et al.*, 1979) or *Streptomyces* (Williams *et al.*, 1983).

Chemical markers are now widely employed in the circumscription of actinomycete genera (see Williams *et al.*, 1989), although numerical taxonomy still provides an effective way of assigning numerous poorly classified organisms to taxospecies (see Goodfellow & Dickinson, 1985). Actinomycetes which contain *meso*-diaminopimelic acid, arabinose and galactose (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970), but lack mycolic acids, form the

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micropolysporas (Goodfellow & Cross, 1984). This suprageneric group accommodates not only the established genera *Actinopolyspora*, *Amycolata*, *Amycolatopsis*, *Faenia*, *Pseudonocardia*, *Saccharomonospora* and *Saccharopolyspora* but also fodder isolates assigned to clusters A and B (Goodfellow *et al.*, 1979). Members of these genera have DNA that is especially rich in guanine (G) plus cytosine (C) and are characterized by a combination of chemical and morphological properties (Goodfellow & Minnikin, 1984; Lechevalier *et al.*, 1986; Embley *et al.*, 1988).

The present study was designed to determine the taxonomic status and affinities of strains belonging to clusters A and B by comparing them, using chemical and numerical taxonomic criteria, with marker strains of established wall chemotype IV taxa lacking mycolic acids. The test strains were isolated from cereal grains, hay and sugar-cane bagasse using half-strength nutrient agar supplemented with cycloheximide and a wind tunnel/Andersen sampler method (Gregory & Lacey, 1963; Lacey, 1971). Isolates assigned to cluster A were most frequently isolated from barley grain and less often from hay and sugar cane bagasse. They were present, together with *Faenia rectivirgula*, in 15% of freshly harvested grain samples but not in grass used to seal moist barley silos. However, after storage, they occurred in 33% of grass and straw samples and in 57% and 32% of grain samples in two seasons (Lacey, 1971). The cluster B isolates chiefly came from hay but they occurred in few samples and were rarely numerous.

METHODS

Strains. The 115 test organisms included representative fodder isolates and reference strains from private and service culture collections (Table 1). Where possible, type strains were included; duplicated cultures of seven strains were used as internal checks on experimental test error. The organisms were maintained on glucose/yeast extract agar (GYEA; Gordon & Mihm, 1962) and as suspensions of spores or mycelial fragments in glycerol (20%, v/v) stored at −20 °C.

Data acquisition. Each strain was examined for 120 unit characters (Table 2) using procedures described previously (Athalye *et al.*, 1985). Unless otherwise stated, tests were incubated at 30 °C and results recorded weekly for up to 28 d apart from those with *Faenia rectivirgula*, which were incubated at 45 °C for 14 d. Tests were repeated only when ambiguous or clearly unexpected results were obtained. Where possible, they were done in Replidishes (Sterilin) (Table 2) which were inoculated, with one exception, from 10- to 14-d-old GYEA master Replidishes using a multipoint inoculation procedure (Sneath & Stevens, 1967). The antibiotic sensitivity tests were inoculated using 7-d-old glucose/yeast extract broth cultures. Inocula for the remaining tests were taken from freshly thawed glycerol suspensions. Data for computation were derived from final test readings apart from the antibiotic sensitivity tests, which were recorded after 5 d; that is, when good growth was apparent on the positive controls.

Coding of data. Nearly all of the characters existed in one of two mutually exclusive states that were scored as plus (1) or minus (0). Qualitative multistate characters, such as pigmentation and colony elevation, were recorded as several independent features scored plus (1) for the character state shown and minus (0) for all other alternatives. The final data matrix contained information on 122 strains and 120 unit characters.

Computation. Data were examined using the CLASP package of programs (written by G. J. S. Ross, F. B. Laukner and D. Hawkins, Rothamsted Experimental Station, Harpenden, UK) on an ICL 470/472 computer, using the simple matching coefficient (S_{SM}; Sokal & Michener, 1958), which includes both positive and negative similarities, and the pattern coefficient (D_P), which allows for differences in growth rates, periods of incubation and similar factors that can distort similarity values (Sneath, 1968). Clustering was achieved using the unweighted pair group method with arithmetic averages (UPGMA) algorithm (Sneath & Sokal, 1973).

Strains and growth conditions for chemosystematic analyses. Biomass for the fatty acid, isoprenoid quinone, polar lipid and wall amino acid analyses was prepared by growing strains representing the *Saccharopolyspora* species (Table 4) in shake flasks of nutrient broth (Oxoid CM3) for 7 to 10 d at 30 °C. Cultures were checked for purity at maximum growth, killed with formalin (1%, v/v), harvested by centrifugation, washed with distilled water and freeze-dried. Three representatives of cluster C, strains A258, A259 and A261, grown under the same conditions were used for diaminopimelic acid analyses. For the DNA base composition studies, strains A65 (cluster A) and A85 (cluster B) were grown in nutrient broth to early exponential phase then harvested by centrifugation.

DNA base composition studies. Harvested biomass was washed with TES buffer (0·05 M-Tris, 0·05 M-NaCl, 0·005 M-EDTA; pH 8·0). The cells were then resuspended in 20 ml TES buffer containing sodium dodecyl sulphate (0·5%, v/v) and proteinase K (Sigma 500 μg ml⁻¹) and disrupted by passage through a pre-cooled French pressure cell at 8000 p.s.i. (55 MPa). The DNA samples were then purified further and the thermal denaturation method used to determine the G + C content of DNA extracted from the test organisms (Marmur, 1961). Determinations were performed in 0·1 × SSC (1 × SSC is 0·15 M-NaCl plus 0·015 M-trisodium citrate). *Escherichia coli* DNA (Sigma) with a G + C content of 51 mol % was used as an internal standard.

Extraction and analysis of fatty acid methyl esters (FAMEs). Dried biomass (30 mg) was degraded by acid methanolysis (Minnikin *et al.*, 1980) and analytical and preparative TLC of the methanolysates was carried out as described previously (O'Donnell *et al.*, 1982*a*). Capillary GC of the FAMEs was performed according to Embley *et al.* (1987). The retention times and relative proportions of each FAME were determined using a Shimadzu CR3A computing integrator. The identity of individual esters was established by comparison of the retention times with those of fatty acids isolated from *Saccharopolyspora hirsuta* ATCC 27875 (Embley *et al.*, 1987).

Extraction and analysis of polar lipids and isoprenoid quinones. The small-scale procedure of Minnikin *et al.* (1984) was used to extract these lipids from biomass samples (about 150 mg). Purified isoprenoid quinones were analysed by mass spectrometry and quantified by reverse-phase HPLC (Collins, 1985). Polar lipid patterns were obtained using published procedures (Minnikin *et al.*, 1984). Choline-positive spots were detected on thin-layer plates using Draggendorf's reagent (Wagner *et al.*, 1961).

Cell wall amino acid analysis. Cell walls and heptafluorobutyrate isobutyl ester derivatives of amino acids were prepared as described by O'Donnell *et al.* (1982*b*) and analysed using capillary GC (Embley *et al.*, 1984). The isomeric forms of diaminopimelic acids were determined by chromatography of cell wall hydrolysates on thin-layer sheets (Eastman Kodak 13255) after Staneck & Roberts (1974). Sugars in whole-organism hydrolysates were analysed as their alditol acetates on a Silar 10C (Altech Associates) capillary column using the simplified method of Englyst & Cummings (1984).

Morphological and growth studies. Additional morphological data on isolates were obtained from cultures growing on half-strength nutrient agar at 25 or 40 °C. Colonies were examined *in situ* on the agar surface using a compound microscope with × 20 and × 40 objectives.

Spores, collected by touching collodion-coated copper grids onto sporulating cultures, were examined without further treatment using a Siemens Eliniskop 1A transmission electron microscope.

Growth temperature ranges were determined by incubating cultures on half-strength nutrient agar at different temperatures between 10 and 60 °C, at 5 °C intervals, and recording growth up to 3 weeks.

RESULTS

Numerical classification

The probability (*p*) of an erroneous test result, as estimated from results with the six duplicated strains (Table 1), averaged 4·1%, equal to an observed S_{SM} between duplicated cultures of just under 92%. Cluster composition was not greatly affected by the statistical tests.

The test strains were assigned to six major (5 to 38 strains), six minor (2 to 3 strains) and ten single-member clusters defined at or above the 0·22 level in the D_P, UPGMA analysis (Table 1, Fig. 1). Most clusters were distinct and homogeneous and were named, where possible, after the type or authentic strains they contained. Clusters 1 to 6, all of which encompassed isolates from fodder, showed a close affinity with the type strain of *Saccharopolyspora hirsuta*. Cluster 7 also contained fodder isolates together with strains received as *Actinomadura madurae* and *Nocardiopsis dassonvillei* (Table 1). The three strains received as *Actinomadura* species (cluster 12) were sharply distinguished from all of the other numerically defined clusters.

Cluster 1 accommodated 14 of the 26 cluster B strains. The remaining organisms provisionally assigned to cluster B were either recovered as single-member clusters or formed minor phena 2, 3, 5 and 6. In contrast, all but one of the 38 cluster A strains constituted cluster 4; the exception, strain A700, formed a single-member cluster. Similarly, clusters 7 and 9 encompassed the cluster C isolates and *Saccharothrix aerocolonigenes*, respectively. *Amycolatopsis orientalis* strains formed clusters 8 and 10, apart from *A. orientalis* N883, which was recovered as a distinct single-member cluster. The organisms labelled *Actinomadura* that formed cluster 12 had properties typical of the genus *Rhodococcus* (Goodfellow, 1986). The affinities of *Amycolata autotrophica* A52 and other strains forming single-member clusters can be seen in Fig. 1.

Cluster composition was not greatly affected when the S_{SM} coefficient was employed, although some of the phena were arranged differently. The clusters were defined at or above the 77% similarity (*S*-) level. Major clusters 1 (*Saccharopolyspora gregorii*) and 7 (cluster C) were recovered in their entirety, as were minor clusters 3 (cluster B), 11 (*Faenia rectivirgula*) and 12 (*Rhodococcus* sp.). Similarly, *Amycolata autotrophica* A52, *Amycolatopsis orientalis* N883, *Saccharopolyspora hirsuta* N745 and fodder isolates A49, A61, A68 and A108 again formed single-member clusters. Cluster 4 (*Saccharopolyspora hordei*) in the S_{SM}, UPGMA analysis

Table 1. *Designations and sources of strains assigned to clusters in the D_P, UPGMA analysis*

Cluster	Laboratory number	Received as	Source‡
1	A44–A46, A79	Cluster B	JL A1248, JL A640, JL A336, JL A1247
	A85[a], N259[a]		JL A333, CUB 355 (JL A333); hay
	A62, A66, N252		JL A66, JL A694, straw; CUB 130, soil
	A53, A60, A81, A86, A89, N258		JL A670, JL A671, JL A637, JL A320, A674, CUB 253 (JL A321); barley
2	A71, N260	Cluster B	JL A548, CUB 359, (JL A409); barley
3	A213, A214	Cluster B	JL A1558, JL A1559; barley
4	A55, A58, A65, A67, A70, A83, A84, A105, A215, A216[b], D7[b], A218, A219, A220, A221, N269	Cluster A	JL A428, JL A471, JL A868, JL A491, JL A305, JL A581, JL A470, JL A474, JL A1560, JL A1561[b], JL A1563, JL A1560, JL A1565, JL A1566, CUB 437 (JL A683); barley
	A54, A63, A64, A87, A91, A266, A267		JL A735, JL A1276, JL A826, JL A1016, JL A620, JL A1234, JL A1549; hay
	A47, A56, A77[c], A104[c], A88		JL A676, JL A894, JL A232[c], JL A824; sugar cane bagasse
	A51[d], N268[d], A48, A59		JL A337, CUB 435 (JL A337), oats; JL A678, grain; JL A795, dust
	A78, A158, A159[e], A238[e], A160[f], A252[f]		JL A789, JL A1618, JL A1619, NCPF 1184 (JL A1619), JL A1620, NCPF 1185 (JL A1620)
5	A72[g], N261[g]	Cluster B	JL A410, CUB 360 (JL A410); barley
6	A57, A75, A90	Cluster B	JL A879, coffee beans; JL A673, barley; JL A272, straw
7	A106, A107, A217, A256, A258, A259, A261, A262, A263	Cluster C	JL A670, JL A888, JL A1562, JL A317, JL A436, JL A455, JL A506, JL A544, JL A624; barley
	A254, A255, A257, A260, A264		JL A204, bagasse; JL A282, JL A331, hay; JL A488, air; JL A1041, maize
	A110	*Actinomadura madurae*	CDC N248; sputum
	A120, A121	*Nocardiopsis dassonvillei*	IMRU 1250, IMET 9653; soil
8	N855, N880, N881, N857, N858, N875, N876, N878	*Amycolatopsis orientalis*	IMRU 1148, IMRU 1627, IMRU 1628 (NRRL B-2452), IMRU 1389, IMRU 1390, IMRU 1131, IMRU 1409, clinical specimens; IMRU 1592, soil
9	N833–N836, N838, N839, N849*, N850, N851, N852, N853	*Saccharothrix aerocolonigenes*	IMRU 550, IMRU 938, IMRU 1106, IMRU 1566 (NRRL B-1125T), IMRU 1593 (CDC W-2104), IMRU 1663, IMRU 1546 (NRRL B-3274), IMRU 1594 (CDC W-2105), IMRU 1599 (CDC W-2158), IMRU 1667, IMRU 1668; soil
	N837, N848, N854		IMRU 1589 (CDC W-1894), spinal fluid; IMRU 1432, IMRU 1700
10	N859–N861, N863*, N879	*Amycolatopsis orientalis*	IMRU 1600 (NRRL B-3891), IMRU 1632, IMRU 1633, ISP 5040 (ATCC 19795), IMRU 1601; soil
11	A162, A163, A164	*Faenia rectivirgula*	JL A1622 (INMI 682), JL A1623 (INMI 1325), JL A1624 (INMI 9553)
12	A103, A135, A136	*Actinomadura* spp.	RIA 452, CCM 5509, CCM 5510

Table 1 *(continued)*

Cluster	Laboratory number	Received as	Source‡
SMC†	A52*	*Amycolata autotrophica*	JL A1269 (IMRU 1595, ATCC 19727)
	N883	*Amycolatopsis orientalis*	IMRU 1644 (CDC W-2648); spinal fluid
	N745*	*Saccharopoly-spora hirsuta*	JL A1143 (ATCC 27875); sugar cane bagasse
	A61, A68, A265	Cluster B	JL A893, bagasse; JL A302, straw; JL A1046, hay
	A700	Cluster A	Laboratory isolate
	A49, A108, N251	Fodder isolates	JL A286, barley; JL A1273, hay; N251, CUB 123

* Type strain.
a–g Duplicated cultures.
† SMC, single-member clusters.
‡ Sources: ATCC, American Type Culture Collection, Rockville, Maryland, USA; CCM, Czechoslovak Collection of Micro-organisms, J. E. Purkyne University, Brno, Czechoslovakia; CDC, Center for Disease Control, Atlanta, Georgia, USA; CUB, Dr T. Cross, School of Biomedical Sciences, University of Bradford, Bradford, UK; IMET, Dr H. Prauser, Institute of Microbiology and Experimental Therapy, Jena, GDR; INMI, Institute of Microbiology, The USSR Academy of Sciences, Moscow, USSR; INRU, Institute of Microbiology, Rutgers University, New Brunswick, New Jersey, USA; NCPF, National Collection of Pathogenic Fungi, Central Public Health Laboratory, Colindale, London, UK; JL, Dr J. Lacey, Rothamsted Experimental Station, Harpenden, UK; NRRL, Northern Regional Research Laboratory, Agricultural Research Division, Peoria, USA; RIA, The USSR Research Institute for Antibiotics, Moscow, USSR; RV, Professor R. Vanbreuseghem, Institute of Tropical Medicine, Antwerp, Belgium.

accommodated 37 strains which were divided between two adjoining subclusters at the 72% S-level. Subcluster 4A contained 34 isolates, including strains A700 and N251 which had previously formed single-member clusters, and subcluster 4B contained strains A51, A53 and A78. The *Saccharothrix aerocolonigenes* strains were also recovered in two subclusters. Subcluster 9A encompassed 12 strains and was sharply separated from subcluster 9B, which contained strains N852 and N853. In contrast, phena 2 (cluster B) and 5 (cluster B) formed a single cluster that also contained strain A78 from phenon 4 (cluster A) and strain A90 from phenon 6 (cluster B). Similarly, the *Amycolatopsis orientalis* strains of clusters 8 and 10 formed a single cluster, apart from strain N883, which was again recovered as a single-member cluster. Phenon 6 (cluster B) contained three organisms, having gained strain A265, previously a single-member cluster.

The properties of the multimembered clusters defined in the D_P, UPGMA analysis are given in Table 2.

Species of *Saccharopolyspora* can be distinguished using a combination of biochemical, morphological, nutritional and physiological features (Table 3).

Morphology

Isolates classified in clusters 1, 2, 3 and 5 were similar in appearance. Colonies were pale yellowish to warm buff and grew over a temperature range of 10–20 to 35–40 °C, mostly at 15 to 35 °C. There was usually extensive fragmentation of the substrate mycelium into rod-shaped and coccoid elements. White aerial mycelium was formed sparsely, if at all, with most produced by cluster 1 isolates, especially at 20 °C. Little was produced below 15 or above 25 °C. Electron microscopy showed mostly vegetative cells. Spores, with a smooth sheath, were only evident in strain A86, which was also atypical in having aerial mycelium with short side branches.

Cluster 4 isolates grew at higher temperatures than those of clusters 1, 2, 3 and 5. The temperature range for growth was 20–30 up to 60 °C. Colonies were colourless to light or warm buff. The substrate mycelium showed some fragmentation into coccoid elements. When
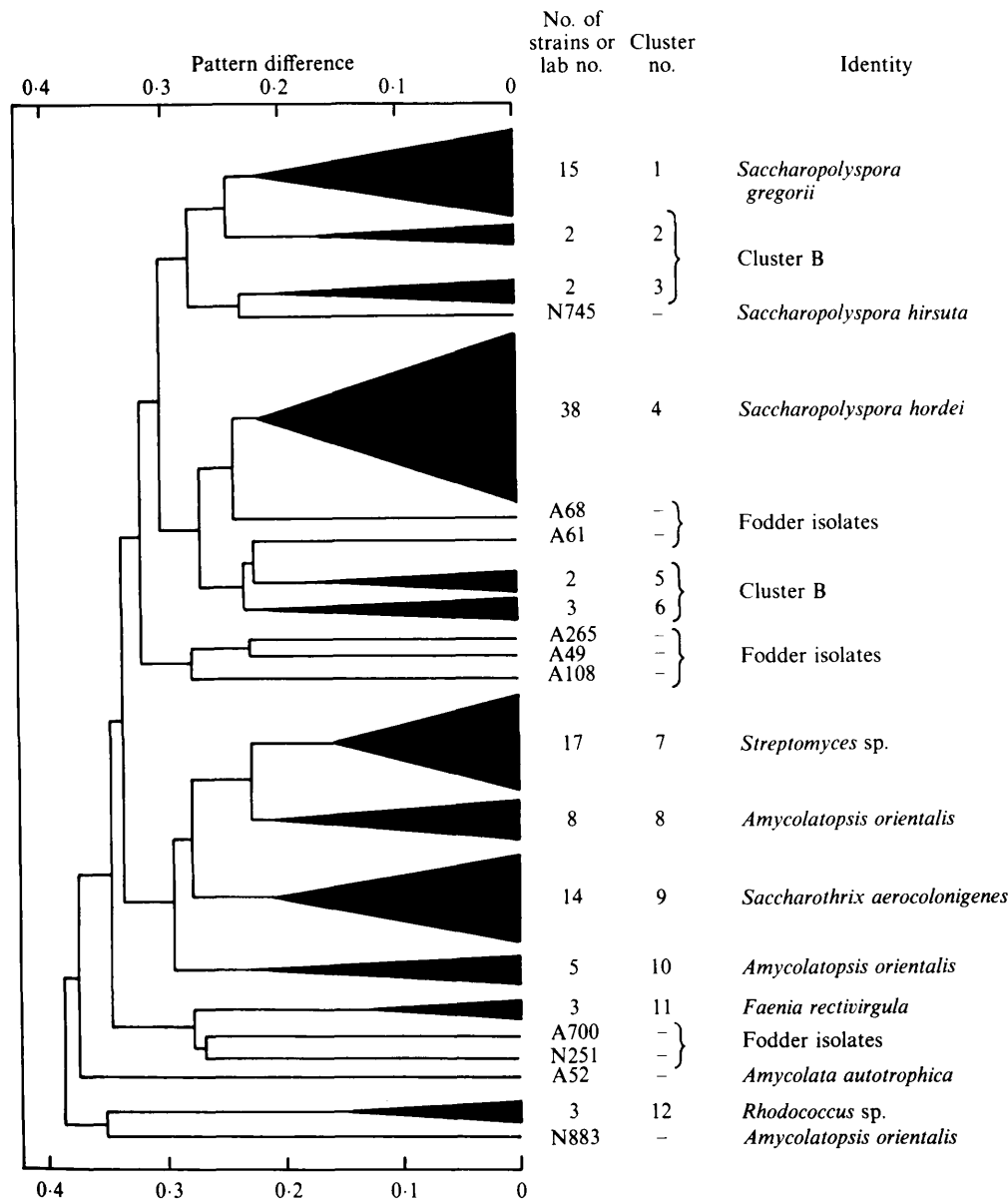
Fig. 1. Simplified dendrogram showing relationships amongst clusters based on the D_P, UPGMA analysis.

produced, aerial mycelium was sparse, white and carried short, flexuous or curved spore chains (Fig. 2). Electron microscopy revealed the spores to be covered with a smooth or slightly warty sheath (Fig. 3) and adjacent spores were often separated by an empty segment of hypha, as observed previously in *Saccharopolyspora hirsuta* (Lacey & Goodfellow, 1975). Isolated spores often had an annulus of sheath material evident at each end (Fig. 4). Strain A65 was atypical in growing only between 20 and 30 °C and in having short, straight branches on aerial hyphae.

Two of the three cluster 6 isolates grew over the range 20 to 60 °C while strain A57 grew only between 15 and 35 °C. Strains A75 and A90 resembled cluster 4 isolates while strain A57 colonies were colourless with little fragmentation or evidence of spores.

Table 2. *Distribution of positive characters to major and minor clusters defined in the D_P, UPGMA analysis*

Values are numbers of strains with positive character states

	Major clusters						Minor clusters					
	Saccharopolyspora gregorii	*Saccharopolyspora hordei*	*Streptomyces* sp.	*Amycolatopsis orientalis*	*Saccharothrix aerocolonigenes*	*Amycolatopsis orientalis*	Cluster B	Cluster B	Cluster B	Cluster B	*Faenia rectivirgula*	*Rhodococcus* sp.
Cluster number ...	1	4	7	8	9	10	2	3	5	6	11	12
Number of strains ...	15	38	17	8	14	5	2	2	2	3	3	3
Biochemical tests:												
Allantoin hydrolysed	0	1	12	6	10	5	1	0	0	0	0	7
Melanin produced	8	3	3	0	5	0	0	1	1	1	0	2
Nitrate reduced	4	11	14	5	10	5	1	2	1	1	1	3
Urea hydrolysed	1	4	14	7	11	5	1	1	0	1	0	2
Colony properties:												
Aerial mycelium												
Sparse	1	0	2	1	3	0	0	0	0	1	3	0
Moderate	0	0	6	5	1	0	0	0	0	1	0	0
Abundant	0	0	3	2	1	1	0	0	0	0	0	0
Colonies												
Brown	0	0	0	0	1	0	0	0	0	0	0	0
Pink/orange	0	0	0	0	0	0	0	0	0	0	0	3
White/cream/yellow	15	37	17	8	14	5	2	2	2	3	3	0
Colony elevation												
Convex	4	17	1	2	1	0	0	0	2	1	0	1
Crateriform	8	20	11	3	0	0	1	0	0	2	3	2
Irregular	3	1	5	3	13	5	1	2	0	0	0	0
Colony margin												
Entire	6	26	0	0	3	0	0	0	0	0	3	3
Filamentous	6	6	0	1	0	0	0	2	0	0	0	0
Highly filamentous	3	6	17	7	12	5	2	0	2	3	0	0
Diffusible pigment												
Brown	0	0	0	0	0	0	0	0	0	0	0	0
Yellow/orange	0	0	0	1	0	0	0	0	0	0	0	0
Degradation of:												
Aesculin	15	38	17	8	14	5	2	1	2	3	3	3
Arbutin	13	37	17	5	11	5	2	1	2	3	0	3
Casein	15	15	17	8	14	5	0	2	2	2	0*	1
DNA	8	36	11	8	13	5	0	2	1	2	0	0
Elastin	13	2*	16	6	14	5	0	1	0	0	0	0
Gelatin	15	22	16	8	14	5	2	2	2	2	0	0
Guanine	4*	35	15	7	14	5	2	2	2	2	3	0
Hypoxanthine	12	27	17	8	14	5	2	2	2	3	0	0
RNA	11	35	16	8	13	5	2	2	2	3	3	3
Starch	15	37	17	7	14	5	2	2	2	3	0	0
Testosterone	5	35	16	8	14	5	2	1	2	3	0	3
Tween 20	15	38	17	7	13	4	2	2	2	3	3	3
Tween 80	14	38	17	8	14	5	2	2	2	3	3	3
L-Tyrosine	13	34	11	8	14	5	2	1	2	3	3*	1
Xanthine	13	34	15	6	0*	2	2	2	2	3	0*	0
Xylan	0	2	0	3	10*	2	0	0	0	0	0	0

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Table 2 (*continued*)

Cluster number ...	1	4	7	8	9	10	2	3	5	6	11	12
Number of strains ...	15	38	17	8	14	5	2	2	2	3	3	3
Growth at * :												
10 °C	7	4	0	3	6	2	2	0	2	2	0	3
20 °C	15	10	17	8	13	5	2	2	2	2	0	3
45 °C	5	37	15	6	5	2	2	2	2	3	3	3
Growth in presence of (% w/v) * :												
Adenine (0·4)	3	16	15	6	0	4	2	0	0	1	0	2
Bismuth citrate (0·0001)	15	37	17	8	14	5	2	2	2	3	3	3
(0·001)	3	8	15	7	13	4	0	2	0	1	0	3
(0·01)	0	1	11	5	5	3	0	1	1	0	0	2
Crystal violet (0·0001)	14	29	17	8	12	5	2	2	1	3	3	3
(0·001)	8	16	15	8	5	4	2	0	1	2	2	2
Phenol (0·01)	15	36	17	8	15	5	2	2	2	3	2	3
(0·1)	11	27	13	7	9	2	1	0	1	0	1	3
Phenyl ethanol (0·1%, v/v)	15	36	17	8	12	5	2	2	2	2	3	3
(0·2%, v/v)	11	26	5	4	1	1	2	2	2	1	2	3
(0·3%, v/v)	7	17	5	2	1	0	1	0	2	1	2	3
(0·4%, v/v)	0	2	2	2	1	0	0	0	0	0	1	0
Potassium tellurite (0·001)	14	36	15	8	12	5	2	2	2	3	1	3
(0·005)	3	15	1	7	11	4	2	2	1	0	1	3
(0·01)	1	6	0	7	0	4	2	2	0	0	0	3
Sodium azide (0·001)	15	33	17	8	11	4	2	2	2	3	3	3
(0·01)	5	3	17	7	0	1	1	0	0	0	0	0
(0·02)	4	1	16	6	0	1	2	0	0	0	0	0
Sodium chloride (3)	15	38	17	8	11	5	2	2	2	3	3	3
(5)	15	38	17	7	10	5	2	2	2	3	3	3
(7)	15	38	17	7	8	4	2	2	2	3	3	3
(10)	15	35	17	5	3	2	2	2	2	3	3	3
(13)	14	23	17	3	0	1	1	2	2	1	1	2
Teepol (0·01)	14	36	17	8	14	2	2	2	2	3	3	3
Tetrazolium (0·01)	15	37	17	8	14	5	2	2	2	3	3	3
(0·1)	11	23	17	8	11	4	0	2	0	3	1	3
Thallous acetate (0·0001)	12	35	17	8	14	5	2	1	2	3	3	3
(0·001)	8	17	10	8	9	2	2	0	1	3	0	3
(0·01)	2	0	1	1	0	0	0	0	0	1	0	0
Growth on sole carbon source at 1% (w/v) * :												
Adonitol	12	20	17	8	12	5	2	1	2	2	2	0
L(+)Arabinose	15	32	17	8	14	5	2	2	2	3	0	3
Cellobiose	7	30	17	8	14	4	2	2	0	3	3	0
Dextrin	4	29	17	8	3	5	2	1	2	2	3	3
D(−)Fructose	15	36	17	7	13	5	2	2	2	3	3	3
D(−)Galactose	15	36	17	6	13	5	2	2	2	3	0	1
D(+)Glucosamine	4	22	17	3	2	4	1	1	2	1	0	0
Glycerol	13	33	17	8	14	5	2	1	2	3	3	0
Glycogen	2*	34	16	8	14	5	2	2	2	1	0	0
Lactose	1	15	17	6	14	5	2	1	2	2	1	0
Maltose	13	35	17	0*	13	5	2	1	2	2	3	1
Mannitol	15	30	17	8	14	5	2	1	2	2	3	0
D(+)Mannose	15	31	17	8	13	5	2	1	2	3	3	3
L(+)Rhamnose	15	29	13	5	14	4	2	2	2	2	2	1
Starch	2*	32	17	2	13	5	2	1	2	3	3	3
Sucrose	14	34	17	8	14	5	2	1	2	1	3	3
Trehalose	3	29	17	8	9	4	2	1	2	2	3	3
Xylose	15	30	17	8	14	5	2	2	2	3	3	2
At 0·1% (w/v):												
D(−)Alanine	12	18	9	2	11	5	2	0	2	1	1	0
L(−)Alanine	12	21	16	4	12	5	2	0	2	1	2	0
Arbutin	1	23	12	5	12	4	2	0	2	3	0	0
L(−)Proline	14	16	17	3	11	5	2	0	2	2	3	0
Protocatechuic acid	6	26	17	8	7	4	2	2	2	3	0	3
L-Serine	3	21	17	4	9	5	2	1	2	3	3	0
Sodium butyrate	10	33	8	5	14	3	2	1	1	3	3	0

Table 2 (*continued*)

Cluster number ...	1	4	7	8	9	10	2	3	5	6	11	12
Number of strains ...	15	38	17	8	14	5	2	2	2	3	3	3
Sodium propionate	13	23	14	4	11	4	1	2	2	1	3	0
Sodium pyruvate	13	26	17	6	13	4	2	1	2	3	1	0
L-Tyrosine	4	27	17	8	10	5	2	0	2	3	2	0
Resistance to antibiotics (µg ml⁻¹)* :												
Cephaloridine hydrochloride (2)	12	28	17	8	9	5	0	0	0	3	3	1
(10)	5	3	16	7	6	5	0	0	0	1	0	0
Demeclocycline (2)	0	0	17	6	0	4	0	0	0	0	3	0
(8)	0	0	15	0	0	0	0	0	0	0	0	0
Gentamicin sulphate (4)	1	27	0	4	0	3	0	0	0	0	3	0
(16)	0	0	0	1	0	1	0	0	0	0	0	0
Lincomycin hydrochloride (16)	12	37	17	8	10	5	2	0	0	3	3	0
(20)	3	32	17	8	4	5	1	0	0	2	3	0
Neomycin sulphate (3)	10	38	13	8	14	5	2	1	2	0	3	3
(10)	1	26	2	7	14	4	0	1	0	0	3	0
Oleandomycin sulphate (2)	13	38	17	8	12	5	2	1	2	3	3	2
(16)	2	30	17	7	4	5	2	0	2	2	2	0
Sodium benzylpenicillin (10)	11	34	17	8	7	5	0	0	0	3	3	1
(20)	5	25	17	0	0	0	0	0	0	3	3	1
Rifampicin (2)	0	0	17	8	0	5	0	0	0	1	0	1
(6)	0	0	17	6	0	4	0	0	0	0	0	0
Streptomycin sulphate (4)	0	0	4	5	0	5	0	0	0	1	0	0
(16)	0	0	0	3	0	1	0	0	0	0	0	0
Tobramycin sulphate (1)	4	37	17	5	1	5	2	0	2	2	3	3
(8)	0	16	2	0	0	0	0	0	0	0	0	0
Vancomycin hydrochloride (0·25)	4	12	16	8	6	5	1	0	0	0	2	1
(2)	0	0	1	8	5	5	0	0	0	0	0	0

* Tests done in Replidishes

Table 3. *Characteristics differentiating Saccharopolyspora species*

Key: +, >80% of isolates positive; −, <20% of strains positive; v, 21–79% ⁻isolates positive; v(−), most stains negative; ND, not determined. Abbreviations: R, red; O, orange; Y, yellow; C, colourless; Bf, buff; Br, brown; G, grey; P, pink; W, white.

| Species ...	*S. hirsuta*†			*S. erythraea*†	*S. gregorii*	*S. hordei*
Subspecies ...	*hirsuta*	*kobensis*	*taberi*			
Substrate mycelium colour	C–Bf	C–Bf–Y–P	C–Y	OY–RBr	C–Bf	C–Bf
Aerial mycelium produced	+	+	−	+	−	−
Aerial mycelium colour	W	W	ND	P–BrG–W	W	W
Soluble pigment colour	Y	Y–R	O–R	Y–POBr	C	C
Spore surface	Hairy	Hairy	ND	Spiny	Smooth	Smooth
Decomposition of:						
Urea	+	ND	−	−	−	−
Elastin	+	+	ND	ND	+	−
Reduction of nitrate	−	+	+	+	v(−)	v(−)
Growth at:						
10 °C	−	−	−	−	v	−
45 °C	+	+	v	−	−	+
50 °C	v	−	−	−	−	+
Utilization of:						
Arabinose	−	−	+	+	+	+
Glycogen	ND	ND	ND	ND	−	+
Lactose	+	ND	v	−	−	v
Rhamnose	+	−	ND	ND	+	v
Starch	+	ND	+	+	−	+
Xylose	+	−	ND	ND	+	v

† Data from Lacey (1989).
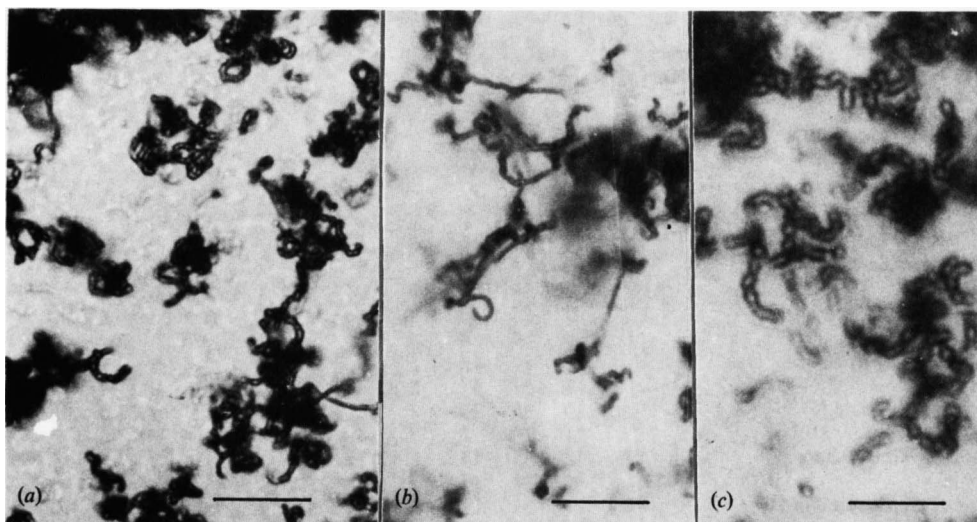
Fig. 2. *Saccharopolyspora hordei*. Sporulation on aerial hyphae. Bars, 20 μm (*a*, *b*) and 10 μm (*c*).

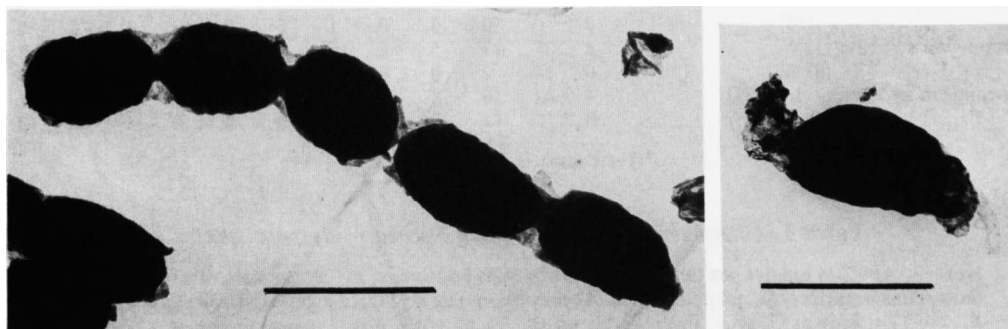Fig. 3   Fig. 4

Fig. 3. *Saccharopolyspora hordei*. Transmission electron micrograph of spore chains. Bar, 1 μm.
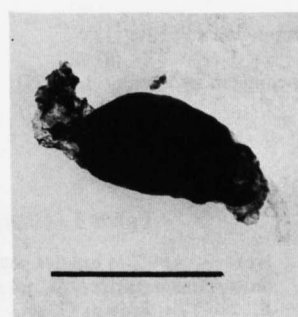Fig. 4. *Saccharopolyspora hordei*. Single spore showing remnants of sheath. Bar, 1 μm.

Table 4. *Percentage fatty acid composition of selected Saccharopolyspora strains*

Strain	*i*-14:0	*i*-15:0	*ai*-15:0	*i*-16:0	17:1$^{\triangle 9}$	16Me	*i*-17:0	*ai*-17:0	*i*-17Me	17Me
S. hirsuta										
ATCC 27875*	tr	15·1	1·2	16·1	1·7	3·5	18·1	14·7	13·1	6·0
S. gregorii (cluster B)										
A60	1·7	4·6	3·4	55·5	1·4	1·2	1·2	19·0	tr	3·9
A85*	2·5	13·5	8·4	27·9	1·5	2·0	5·7	21·1	2·2	9·8
N259	tr	15·7	5·3	17·7	tr	3·3	11·3	33·2	tr	6·7
S. hordei (cluster A)										
A54*	tr	5·6	1·3	33·8	tr	1·0	17·2	21·1	1·5	10·2
A65	tr	4·1	2·0	32·2	3·3	tr	14·5	35·1	tr	1·0
A215	tr	14·3	2·4	35·6	1·0	1·0	16·6	21·5	tr	tr

The header row above has a spanning header "Assignment†:" over the fatty acid columns.

* Type strain.
† Trace amounts of *i*-16$^{\triangle 9}$, 15Me, 16:1$^{\triangle 9}$, 16:0, 17:0, 18Me and a partially characterized dimethyl branched fatty acid were also detected. Abbreviations for the FAMEs are explained by the following examples: 16:0, straight-chain hexadecanoic acid; *i*-15:0, 13-methyltetradecanoic acid; *ai*-15:0, 12-methyltetradecanoic acid; *i*-16:1$^{\triangle 9}$, 14-methyl-*cis*-9,10-pentadecenoic acid; 16:1$^{\triangle 9}$, *cis*-9,10-hexadecenoic acid; 15Me, 10-methylhexadecanoic acid; *i*-17Me, 10,15-dimethylhexadecanoic acid.
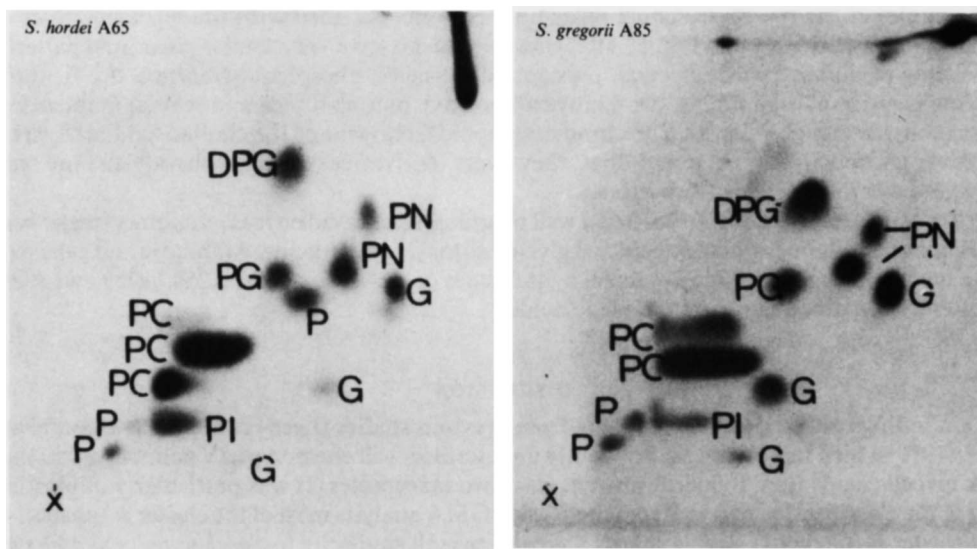
Fig. 5. Two-dimensional thin-layer chromatograms of polar lipids of *Saccharopolyspora hordei* A65 and *Saccharopolyspora gregorii* A85. DPG, diphosphatidylglycerol; G, glycolipids; P, phospholipids; PC, choline-positive phospholipids; PG, phosphatidylglycerol; PI, phosphatidylinositol; PN, ninhydrin-positive phospholipids; X, origin.

Cluster 7 isolates differed from those assigned to other clusters in having a well-developed aerial mycelium that was white except at optimum temperatures, when it assumed a pinkish tinge (2ca, red series; Tresner & Backus, 1963). The colony reverse was white to pale yellow. Spore chains were produced on the aerial mycelium in coils and spirals except in strain A254, where they were close to flexuous. Spores were smooth. The temperature range for growth was 20–25 to 40 °C but strain A254 grew up to 55 °C.

Of the single-member clusters, organism A61 was typical of the appearance of *Faenia rectivirgula* and grew at temperatures from 35 to 60 °C. Strain A68 resembled cluster 4 isolates. Organisms A215 and A265 resembled cluster 1 strains, but without aerial mycelium and with little fragmentation.

Chemosystematic analyses

The mean DNA base composition values derived from the two determinations performed on strains A65 (cluster 4) and A85 (cluster 1) were 72 and 74 mol % G+C, respectively.

TLC analysis of whole-organism methanolysates of the test strains of *Saccharopolyspora* (Table 4) revealed major components (R_F 0·8) that corresponded to non-hydroxylated FAMEs (Minnikin *et al.*, 1980). These strains also contained components that co-chromatographed with the methyl ester of 2-hydroxystearate (R_F 0·2). All the organisms tested gave qualitatively similar fatty acid profiles (Table 4) that consisted of *iso*-, *anteiso*- and 10-methyl branched saturated, *iso*- branched mono-unsaturated, and straight-chain saturated and mono-unsaturated fatty acids. The major components were *iso*-15, *anteiso*-15, *iso*-16, *iso*-17, *anteiso*-17 and 17Me, although the relative proportions of these compounds varied between strains. The *S. gregorii* and *S. hordei* strains contained small amounts of *iso*-10-methylhexadecanoic acid (<2·2%) whereas *S. hirsuta* ATCC 27875 possessed a relatively large proportion of this compound (13·1%). Mycolic acids were not detected.

Components that co-chromatographed with vitamin K_1 and gave mass spectra characteristic for menaquinones were the only isoprenoid quinones found in the test strains. All isolates contained tetrahydrogenated menaquinones with nine isoprene units [MK-9(H_4)] as the major isoprenologue (60–100%) and smaller proportions of MK-8(H_4) (0–5%), MK-9(H_2) (0–30%) and MK-9(H_6) (0–13%).

Examples of the two-dimensional polar lipid patterns obtained with the representatives of clusters 1 and 4 are shown in Fig. 5. All the test organisms gave very similar polar lipid patterns consisting of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two or three choline-positive phospholipids, two ninhydrin-positive phospholipids and several unidentified phospholipids and glycolipids. The chromatographic behaviour of the choline- and ninhydrin-positive phospholipids suggested that they were derivatives of phosphatidylcholine and phosphatidylethanolamine, respectively.

All of the cluster A and B strains had a wall peptidoglycan based on *meso*-diaminopimelic acid and contained alanine, glutamic acid and glycine as major amino acids. Arabinose and galactose were the major sugars. Whole-organism hydrolysates of cluster C strains A258, A259 and A261 (cluster 7) contained LL-diaminopimelic acid.

DISCUSSION

Our findings confirm and extend those from previous studies (Lacey *et al.*, 1978; Goodfellow *et al.*, 1979) which indicated that previously undescribed wall chemotype IV actinomycetes that lack mycolic acids from fodder formed at least two taxospecies. It was particularly interesting that in the classification derived from the D_P, UPGMA analysis most of the cluster A (phenon 4) and cluster B (phenon 1) strains shared a greater overall similarity to *Saccharopolyspora hirsuta* than to *Amycolata autotrophica*, *Amycolatopsis orientalis* and *Faenia rectivirgula*. Indeed, the fodder isolates and *Saccharopolyspora hirsuta* N745 formed a distinct aggregate group. A second, albeit chemically heterogeneous, assemblage encompassed *Amycolatopsis orientalis*, *Saccharothrix aerocolonigenes* (wall chemotype III) and cluster C (wall chemotype I) strains. The latter have properties consistent with their assignment to the genus *Streptomyces* (Williams *et al.*, 1983).

Cluster composition was not markedly affected by the statistics used, or by the 4·1 % test error. However, relationships between clusters were influenced by the coefficients of association. Thus, in the S_{SM}, UPGMA analysis, phenon 1 (cluster B) and *Saccharopolyspora hirsuta* N745 had a closer relationship to the *Amycolatopsis*, *Saccharothrix* and cluster C (*Streptomyces* sp.) strains than to fodder isolates assigned to phenon 4 (cluster A). This and earlier observations (Goodfellow *et al.*, 1979, 1985; Athalye *et al.*, 1985) emphasize the importance of evaluating the composition of numerically circumscribed aggregate groups using data derived from chemical and molecular systematic studies.

A combination of chemical and morphological properties are currently used to classify wall chemotype IV actinomycetes that lack mycolic acids (Goodfellow & Minnikin, 1984; Lechevalier *et al.*, 1986; Embley *et al.*, 1988). The representatives of clusters A (phenon 4) and B (phenon 1) were rich in *iso*- and *anteiso*- fatty acids and contained small amounts of the diagnostic component, *iso*-10 methylhexadecanoic acid. These organisms also contained tetrahydrogenated menaquinones with nine isoprene units as the predominant isoprenologue and had a polar lipid pattern that included major amounts of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and derivatives of phosphatidylcholine and phosphatidylethanolamine. It is evident given these chemical data that phena 1 and 4 are more closely related to *Faenia* and *Saccharopolyspora* than to the genera *Actinopolyspora*, *Amycolata*, *Amycolatopsis*, *Pseudonocardia* and *Saccharomonospora* (Embley *et al.*, 1988). The chemical data recorded for *Saccharopolyspora hirsuta* N745 are in good agreement with previous findings (Embley *et al.*, 1987, 1988).

The genera *Faenia* and *Saccharopolyspora* are currently distinguished by their DNA base composition (Poschner *et al.*, 1985; Lechevalier *et al.*, 1986; Labeda, 1987) and detailed polar lipid profiles (Embley *et al.*, 1988). *Faenia* strains have DNA with relatively low G+C values (66–68 mol %), a single phosphatidylcholine component and many glycolipids; *Saccharopolyspora* strains have DNA richer in G+C (70–77 mol %), multiple phosphatidylcholine components and fewer glycolipids. On this basis the fodder isolates belonging to phena 1 and 4 have more in common with *Saccharopolyspora* than *Faenia*. Ribosomal RNA sequencing data support the association between the genus *Saccharopolyspora* and phenon 4 (Embley *et al.*, 1989).

It is evident from the chemical, morphological and numerical phenetic data that the strains assigned to phena 1 (cluster B) and 4 (cluster A) merit species status within the genus *Saccharopolyspora* Lacey and Goodfellow 1975. Accordingly, the species *Saccharopolyspora gregorii* and *Saccharopolyspora hordei* are proposed for phena 1 and 4, respectively. The minor phena and single-member clusters accommodating the remaining cluster A and B strains may form the nuclei of additional species, but further studies are needed to establish this.

Description of *Saccharopolyspora gregorii* Goodfellow, Lacey, Athalye, Embley and Bowen sp. nov.

gre.gor'i.i. ML gen.n. *gregorii* of Gregory; named after P. H. Gregory, a British mycologist and aerobiologist.

Colonies pale yellowish to buff with sparse white aerial mycelium at growth temperatures of about 20 °C. Substrate mycelium well developed but fragmenting extensively into coccoid elements. Sporulation or fragmentation of the aerial mycelium may sometimes be seen, forming hooks or flexuous chains. Transmission electron micrographs show mainly vegetative rods and cocci but occasionally spores with a smooth sheath. Degrades aesculin, arbutin, casein, elastin, gelatin, hypoxanthine, starch, Tweens 20 and 80, tyrosine and xanthine; utilizes adonitol, L-arabinose, fructose, galactose, glycerol, maltose, mannitol, mannose, rhamnose, sucrose, xylose, D- and L-alanine, L-proline, sodium propionate and sodium pyruvate as sole carbon sources but not glycogen, lactose, starch, trehalose or L-serine. Nitrate is not reduced and neither allantoin nor urea is hydrolysed. The organism is tolerant to a range of antibiotics and chemical inhibitors (see Table 2). Temperature range for growth: 10 to 35 °C.

The wall peptidoglycan contains alanine, glutamic acid and glycine with *meso-* diaminopimelic acid as the diamino acid. The principal wall sugars are arabinose and galactose. The organism is rich in *iso-* and *anteiso-* fatty acids, with 14-methylpentadecanoic and 14-methylhexadecanoic acids predominating. It also contains tetrahydrogenated menaquinones with nine isoprene units as the major isoprenologue and major amounts of diphosphatidyl-glycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol and uncharacterized phospho- and glycolipids. The mol % G + C content of the DNA of the type strain is 74 (T_m).

Isolated from grass, hay, straw, barley grain and soil.

Type strain: *Saccharopolyspora gregorii* NCIB 12823 (A85).

Description of *Saccharopolyspora hordei* Goodfellow, Lacey, Athalye, Embley and Bowen

hor'.de.i. L neut. n. *hordeum*, barley; ML n. *Hordeum*, a genus of grass.

Colonies colourless to light buff, conical, rounded or wrinkled. Substrate mycelium well developed, sometimes fragmenting into coccoid elements. Aerial mycelium lacking or sparse white with short flexuous, hooked or spiral spore chains often with a beaded appearance. Transmission electron micrographs show the spores to be characteristically covered by a smooth sheath, with adjacent spores often separated by a short length of empty hypha. Degrades aesculin, arbutin, DNA, guanine, RNA, starch, testosterone, Tweens 20 and 80, L-tyrosine and xanthine; utilizes L-arabinose, cellobiose, fructose, galactose, glycogen, glycerol, maltose, mannitol, mannose, starch, sucrose and xylose as sole carbon sources. Neither allantoin nor urea is hydrolysed. Tolerant to a range of antibiotics and chemical inhibitors (see Table 2). Temperature range for growth is 20–30 °C up to 60 °C.

The wall peptidoglycan contains alanine, glutamic acid and glycine with *meso*-diaminopimelic acid as the diamino acid. The principal wall sugars are arabinose and galactose. The organism is rich in *iso-* and *anteiso-* fatty acids, with 14-methylhexadecanoic acid and 14- and 15-methylpentadecanoic acids predominating. It also contains tetrahydrogenated menaquinones with nine isoprene units as the major isoprenologue and major amounts of diphosphatidyl-glycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol and uncharacterized phospho- and glycolipids. The mol % G + C content of the DNA of the type strain is 72 (T_m).

Isolated from barley and oat grains, grass hay, straw and sugar cane bagasse.

Type strain: *Saccharopolyspora hordei* NCIB 12824 (A54).

Saccharopolyspora gregorii and *Saccharopolyspora hordei* can be distinguished from one another and from *Saccharopolyspora hirsuta* and *Saccharopolyspora erythraea* by biochemical, morphological, nutritional and physiological properties. Further work is needed to determine whether *Saccharopolyspora* species are implicated in extrinsic allergic alveolitis.

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