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A Novel Actinomycete from Sugar-cane Bagasse: *Saccharopolyspora hirsuta* gen. et sp. nov.

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SUMMARY

A new species of nocardioform actinomycete isolated from spontaneously heated sugar-cane bagasse is described as *Saccharopolyspora hirsuta* gen. et sp. nov. It has affinities with species of both Nocardia and Actinomadura but can be distinguished from both genera by its morphology, sporulation, wall and lipid analyses, antibiotic resistance, degradation and carbon utilization tests.

INTRODUCTION

Sugar-cane bagasse is the squashed chopped fibre left after sugar is extracted from the cane. Initially it contains 50 % water and 3 to 5 % sugar. When stacked in bales it heats rapidly, which favours the growth of thermophilic fungi and actinomycetes (Lacey, 1974). One such actinomycete, *Thermoactinomyces sacchari*, can cause the respiratory disease bagassosis, a form of extrinsic allergic alveolitis (Lacey, 1971*b*). Often, *T. sacchari* was accompanied by an actinomycete which grew well at 40 °C to produce white aerial mycelium, with bead-like chains of spores enclosed in a characteristic hairy sheath. This unidentified actinomycete had fragmenting mycelium and type IV walls (Becker, Lechevalier & Lechevalier, 1965), and degraded casein, tyrosine, hypoxanthine and xanthine; it was therefore assigned provisionally to the genus *Nocardia* (Lacey, 1974). However, isolates have now been compared with representative cultures of allied taxa and seem to represent a homogeneous and distinct taxon. In this paper we describe properties of this organism and propose the name *Saccharopolyspora hirsuta*.

METHODS

Cultures. Colonies of *Saccharopolyspora hirsuta* were isolated from airborne bagasse dust by using a wind-tunnel technique (Lacey, 1971*a*, 1974); the Andersen sampler was loaded with Petri dishes containing half-strength nutrient agar medium with 50 μg actidione/ml (Gregory & Lacey, 1963). Twenty-five *S. hirsuta* isolates from West Indian bagasse samples were compared with cultures representing the genera *Nocardia*, *Actinomadura*, *Mycobacterium* and '*Mycobacterium*' *rhodochrous*. Detailed histories of most of these strains have been given (Goodfellow, 1971; Goodfellow, Fleming & Sackin, 1972; Goodfellow & Orchard, 1974; Goodfellow *et al.* 1974).

All cultures were maintained on yeast extract–malt extract agar (Pridham *et al.* 1957) or V–8 vegetable juice agar (Corbaz, Gregory & Lacey, 1963).

Morphological examination. Colonies growing on agar plates were examined microscopically, and substrate and aerial mycelium were classified as by Cross, Maciver & Lacey (1968). Growth was compared on glycerol–asparagine (Pridham & Lyons, 1961), half-strength nutrient, V–8 juice, and yeast extract–malt extract agars after incubation at 37 or 40 °C. Colour descriptions are those of Ridgway (1912).

Electron microscopy. Spores were examined by transmission and scanning electron microscopy. Spores and hyphae for sectioning were grown on cellophane (Lacey & Vince, 1971) on half-strength nutrient agar. Portions of cellophane, bearing growth, were removed and fixed intact for 5 h in either 2·5 % (v/v) glutaraldehyde or 4 % (v/v) formaldehyde, both in 0·05 M-cacodylate buffer at pH 7, rinsed in buffer alone, then placed in 2 % (w/v) osmium tetroxide overnight. After rinsing in buffer, they were dehydrated in acetone and embedded in Epon. Sections were stained with 2 % (w/v) uranyl acetate followed by Reynold's lead citrate.

Degradation tests. Cultures were examined by using the methods of Goodfellow (1971) with one addition. A dense inoculum of each culture was streaked across a plate containing 0·3 % (w/v) elastin (Sigma) in the basal medium described by Gordon (1967). After 7 and 14 days' incubation, each plate was observed for the disappearance of the insoluble elastin underneath and around the growth.

Antibiotic sensitivity studies. Organisms were tested for their *in vitro* susceptibility to 12 anti-microbial agents (Table 2) by using an impregnated filter-paper disc method (Goodfellow & Orchard, 1974).

Organic compounds as sole sources of carbon. Test strains were examined for their ability to grow on 37 carbon sources (Table 3) by using the media and methods described by Goodfellow (1971).

Analysis for lipid LCN-A. Cultures grown on modified Sauton's medium were analysed for lipid LCN–A (Mordarska, Mordarski & Goodfellow, 1972). These lipids, characteristic of Nocardia species and '*Mycobacterium*' *rhodochrous*, have been shown to be free nocardomycolic acids (Goodfellow *et al.* 1973; Minnikin, Patel & Goodfellow, 1974). Whole organism methanolysates of four isolates from bagasse were also examined for the presence of bound nocardomycolic acids and mycolic acids *sensu stricto* (i.e. characteristic of *Mycobacterium*; Lechevalier, Lechevalier & Horan, 1973).

RESULTS

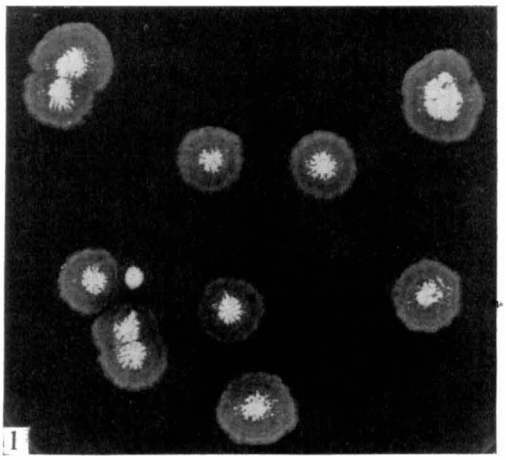
Isolates from bagasse differed little from one another in appearance, wall composition, antibiotic resistance, degradation and carbon utilization tests, but were distinct from all other species with which they were compared. The differences were sufficiently great to necessitate the creation of a new genus, *Saccharopolyspora*, containing the one species *S. hirsuta*. These new taxa are described below.

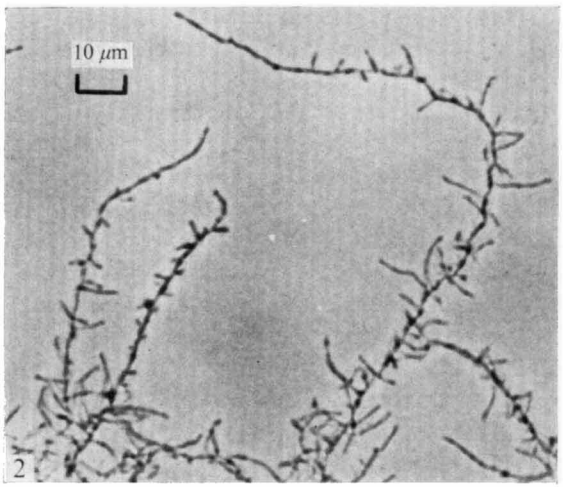
Saccharopolyspora Lacey & Goodfellow gen. nov.

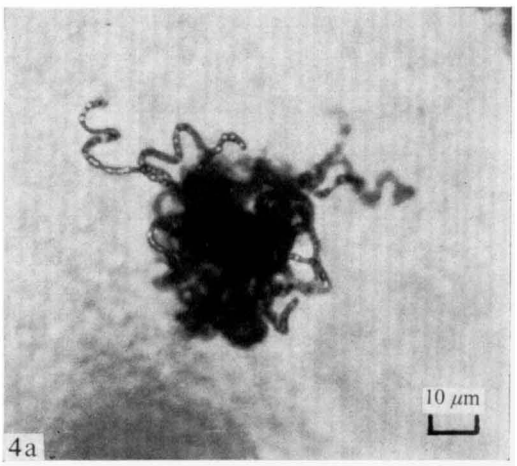
Aerobic, Gram-positive, non-acid-fast actinomycetes with fragmenting substrate mycelium. Aerial mycelium segmenting into spores contained within a sheath. Wall composition type IV (Becker *et al.* 1965).

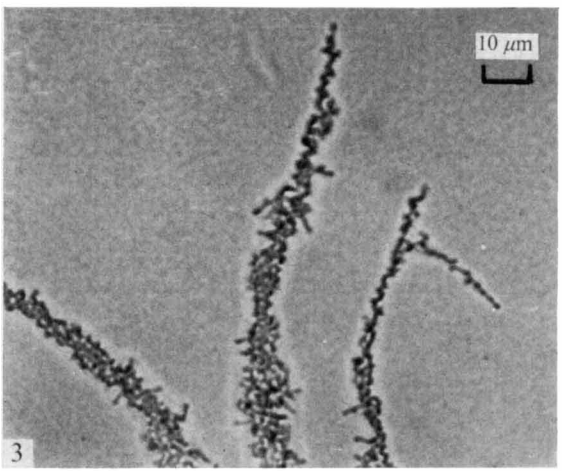
Saccharum, referring to the origin of isolates from sugar cane bagasse; *polyspora*, many spored.

Type species: *Saccharopolyspora hirsuta* Lacey & Goodfellow sp. nov.









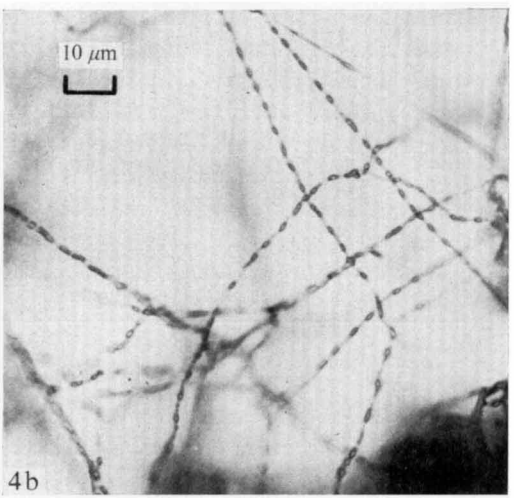


Fig. 1. Colonies of *S. hirsuta*. Half-strength nutrient agar, incubation 37 °C.

Fig. 2. Morphology of substrate mycelium. Glycerol–asparagine agar, incubation 40 °C.

Fig. 3. Fragmentation of substrate mycelium. Glycerol–asparagine agar, incubation 40 °C.

Fig. 4. Spore chains on aerial mycelium showing tufted appearance, and (a) typical curved chains (half-strength nutrient agar, incubation 40 °C) and (b) typical straight chains (V–8 juice agar, incubation 37 °C) sometimes found.

Description of Saccharopolyspora hirsuta Lacey & Goodfellow sp. nov.

Hirsuta, hairy, referring to the spore surface characteristic.

Type strain A1143.

Colonies were thin, raised or convex and usually slightly wrinkled. The substrate mycelium was colourless to cartridge buff and usually mucoid or gelatinous. Sparse white aerial mycelium was produced in tufts at the centres of the colonies (Fig. 1). A yellow soluble pigment was produced on yeast extract–malt extract agar.

Substrate hyphae were 0·4 to 0·6 μm diam, branched, and usually unfragmented at the edge of the colony (Fig. 2). Occasionally, typical nocardioform fragmentation into rod-shaped elements was found at the growing margin (Fig. 3) and more often in older parts of the colony. The substrate hyphae occasionally resembled sporing aerial hyphae, perhaps because aerial hyphae had collapsed on to the surface of the colony.

Aerial hyphae were 0·5 to 0·7 μm in diameter, and characteristically segmented into bead-like chains of spores often separated by lengths of 'empty' hyphae most closely resembling those of *Actinomadura dassonvillei* (Brocq Rousseau) Lechevalier & Lechevalier. Usually the spore chains formed loops or loose spirals, but sometimes long straight chains were found between tufts of aerial mycelium (Fig. 4a, b).

Spores were round to oval, 0·7–1·3 × 0·5–0·7 μm, and covered by a sheath carrying tufts of long straight or curved hairs (Fig. 5a). The morphology of the hairs is better seen on lengths of empty sheath (Fig. 5b) or by scanning electron microscopy (Fig. 6). The sheath surface between the tufts of hairs appeared smooth.

Fine structure. Hyphae (Fig. 7) were bounded by a wall 22 to 30 nm thick. The cytoplasm, enclosed in a typical unit membrane, was granular with diffuse, axial nuclear material. Electron-transparent vacuoles, up to 0·3 μm diam and resembling lipid accumulations in other nocardioform actinomycetes, were sometimes abundant, together with occasional electron-dense granules, up to 0·1 μm diam, resembling polyphosphate or metachromatic granules. Lamellar mesosomes up to 0·25 μm diam were sometimes seen. Septation occurred by a double ingrowth of the wall leading to fragmentation.

Spores were contained within a sheath 18 to 36 nm thick, carrying tufts of structureless hairs. These tufts were triangular at the base (0·2 to 0·3 μm across) extending into an apical filament about 20 nm diam. Occasionally there was unidentified material (Fig. 8) within the sheath between spores, but this area usually appeared empty. The walls of spores were uniformly thickened to 50 to 60 nm, but their internal structure was similar to hyphae except that there were few vacuoles (Fig. 9).

Temperature requirements. Most isolates grew at 25 and 50 °C with an optimum at about 37 to 40 °C. No growth occurred at 10 °C. Most aerial mycelium was produced close to the optimum.

Hydrolysis tests. Saccharopolyspora hirsuta isolates gave positive reactions in most tests

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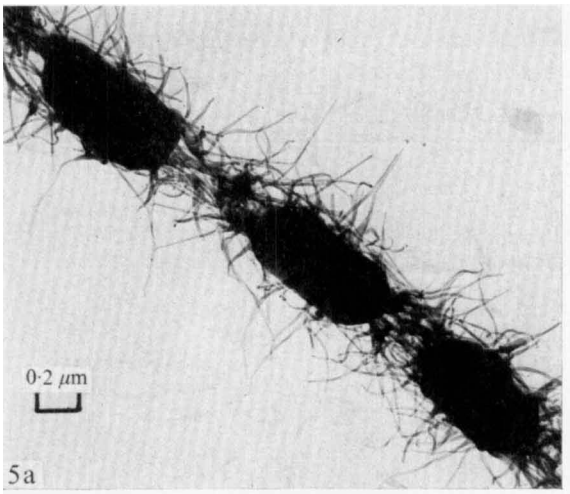
Fig. 5. Electron micrographs of (a) spore chains and (b) empty sheath showing tufted production of hairs.

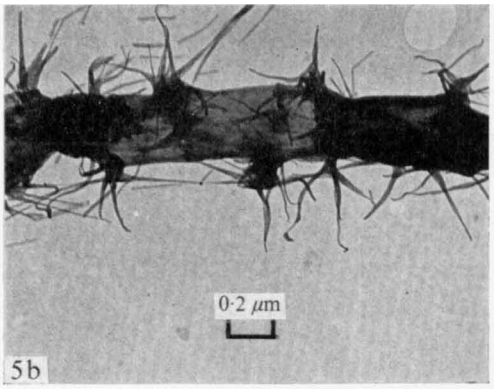
Fig. 6. Scanning electron micrograph of spore chain.

Fig. 7. Longitudinal section of hypha, showing possible lipid accumulations (L) and polyphosphate granules (P).

Fig. 8. Longitudinal section of spore chain showing spores apparently separated by cytoplasm-like material.

Fig. 9. Longitudinal section of mature spore chain showing sheath and hair bases.


0·2 μm
5a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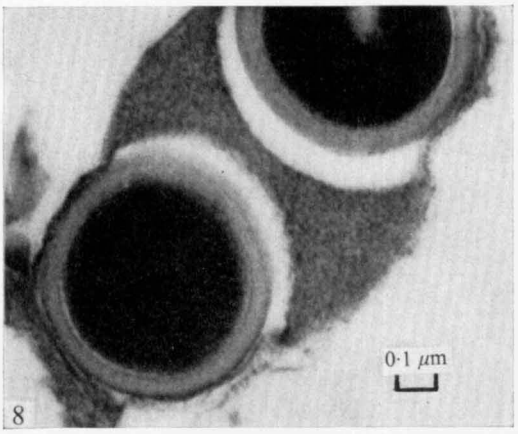
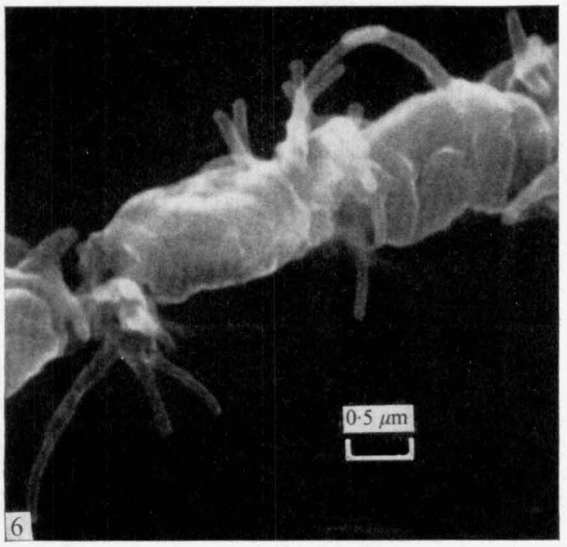

0·1 μm
8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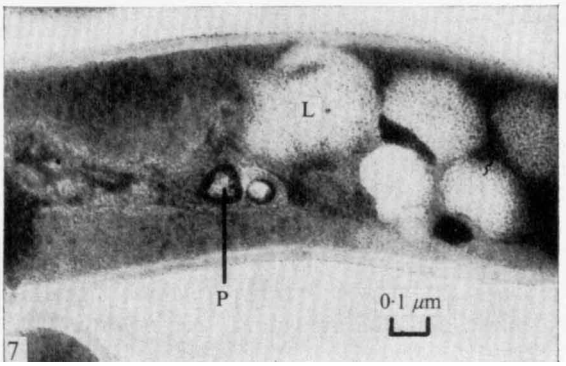
6


L
P
0·1 μm
7

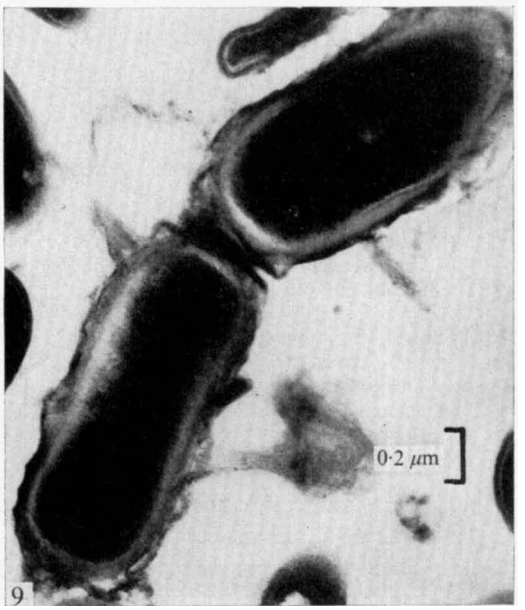

0·2 μm
9

Table 1. *Abilities of Saccharopolyspora hirsuta and other nocardioform taxa to degrade different substrates*

	Percentage of isolates degrading substrate								
	Nocardia asteroides	*N. brasiliensis*	*N. caviae*	*Saccharopolyspora hirsuta*	*Actinomadura dassonvillei*	*A. madurae*	*A. pelletieri*	*'Mycobacterium' rhodochrous*	*Mycobacterium*
Total isolates examined ...	81	18	15	25	6	22	11	146	64
Substrate									
Adenine	0	0	0	100	100	0	0	49	0
Aesculin	100	100	100	100	0	100	0	90	62
Casein	1	100	0	100	81	100	0	0	2
Elastin	0	100	0	100	100	100	100	0	0
Hypoxanthine	0	100	93	100	100	81	100	0	0
Keratin	0	100	0	100	100	81	100	0	0
Tyrosine	1	94	0	100	100	90	100	60	0
Urea	98	100	100	100	33	0	9	75	66
Xanthine	0	0	100	100	100	0	0	0	0
Xylan	0	0	0	0	0	0	0	0	0
Resistance to lysozyme (%)	100	100	100	0	0	0	0	14	14

Table 2. *Susceptibility of Saccharopolyspora hirsuta and other nocardioform taxa to anti-microbial agents impregnated in filter-paper discs*

[Filter-paper discs were soaked in anti-microbial agent at the concentrations (μg/ml) given in parentheses.

	Percentage susceptible							
	Nocardia asteroides	*N. brasiliensis*	*N. caviae*	*S. hirsuta*	*'Mycobacterium' rhodochrous*	*Actinomadura dassonvillei*	*A. madurae*	*A. pelletieri*
Total isolates examined ...	30	28	15	25	38	5	5	5
Gentamycin (100)	80	100	100	0	97	100	0	100
Kanamycin (10)	3	0	80	0	36	0	0	20
Streptomycin (100)	17	11	0	0	94	100	100	40
Neomycin (50)	76	11	20	0	94	100	100	40
Tobramycin (100)	69	86	59	0	94	100	100	100
Rifampicin (50)	0	0	7	8	91	0	0	20
Erythromycin (50)	43	54	20	0	83	0	0	20
Fusidic acid (100)	40	0	0	84	91	0	0	40
Minocycline (50)	33	25	73	80	70	0	0	0
Vancomycin (50)	13	4	0	44	100	0	0	0
Dapsone (500)	23	36	20	92	26	0	0	20
Septrin (500)	43	76	33	100	36	0	0	0

(Table 1) and can readily be distinguished from Nocardia species by their ability to degrade adenine and their susceptibility to lysozyme. Degradation of elastin by *S. hirsuta* suggests possible pathogenic properties since all Nocardia and Actinomadura species with this ability can cause mycetoma.

Antibiotic-sensitivity studies. The antibiotic-sensitivity pattern distinguished *S. hirsuta* from the other nocardioform taxa studied (Table 2). *Saccharopolyspora hirsuta* showed

Table 3. *Suitability of sole carbon sources for energy and growth*

	Percentage of isolates growing on carbon source								
	Nocardia asteroides	*N. brasiliensis*	*N. caviae*	*S. hirsuta*	*Actinomadura dassonvillei*	*A. madurae*	*A. pelletieri*	'*Mycobacterium*' *rhodochrous*	*Mycobacterium*
Total isolates examined ...	81	18	15	25	6	22	11	146	64
Adonitol	0	0	0	100	0	72	0	0	0
Arabinose	0	0	0	0	66	59	63	1	59
Cellobiose	8	0	0	100	83	14	0	0	20
Erythritol	0	0	0	100	0	0	0	0	—
Fructose	96	91	100	100	100	59	0	99	77
Galactose	20	91	0	100	66	68	63	1	21
Glucose	99	100	100	100	100	100	100	100	97
Glycerol	92	94	93	100	100	72	45	98	72
Inositol	4	93	93	100	33	0	0	8	56
Lactose	0	0	0	100	0	0	0	0	2
Maltose	60	55	13	100	100	77	18	84	56
Mannitol	37	84	100	100	100	72	9	87	75
Mannose	48	66	25	100	100	50	9	98	80
Melezitose	0	0	0	0	0	0	0	6	0
α-Methyl-D-glucoside	0	0	0	100	17	0	0	0	—
β-Methyl-D-glucoside	7	6	93	100	—	—	—	4	—
Raffinose	0	0	0	100	0	0	0	0	0
Rhamnose	30	0	0	100	66	72	9	0	38
Salicin	12	18	13	0	33	0	0	27	0
Sorbitol	3	0	0	100	0	14	0	85	35
Sucrose	49	83	50	100	100	14	0	98	34
Trehalose	25	100	27	100	33	95	90	88	41
Xylose	4	0	0	100	83	90	0	1	53
Sodium acetate	100	100	100	100	100	81	100	99	80
Sodium benzoate	10	0	0	100	0	0	0	69	44
Sodium butyrate	100	100	100	100	100	72	100	96	80
Sodium citrate	44	88	20	100	100	45	90	88	32
Sodium fumarate	84	44	100	100	—	—	—	94	—
Sodium H-malate	100	100	100	100	100	68	100	96	60
Sodium propionate	96	100	100	80	83	68	100	97	85
Sodium pyruvate	96	100	100	88	83	50	100	90	24
Sodium succinate	92	88	100	100	100	81	63	97	3
Sodium tartrate	0	0	0	0	0	0	0	15	0
Adipic acid	2	0	0	0	0	0	0	88	6
Sebacic acid	96	100	100	100	83	0	90	80	42
Testosterone	82	0	100	100	33	0	0	71	32

remarkable resistance to the anti-microbials tested, except large concentrations of septrin, dapsone, minocycline and fusidic acid.

Organic compounds as sole sources of carbon for energy and growth. Saccharopolyspora hirsuta isolates were extremely versatile and utilized 32 of 37 carbon sources tested (Table 3). The pattern of utilization distinguished them from other taxa.

Analysis for lipid LCN-A. Lipid LCN-A was detected in all of the Nocardia and '*Mycobacterium*' *rhodochrous* cultures tested but not in *S. hirsuta*, *Mycobacterium* spp. or *Actinomadura* spp. Bound mycolic acids were not detected in the four cultures of *S. hirsuta* examined.

Differentiation from related taxa. Table 4 lists characters by which *S. hirsuta* may be

Table 4. *Differentiation of Saccharopolyspora from allied taxa*

	Nocardia	*Saccharo-polyspora*	*Actinomadura*	'*Myco-bacterium*' *rhodochrous*	*Myco-bacterium*
Morphological characters					
Aerial hyphae	+	+	+	−	−
Arthrospores	±*	+	±	−	−
Spore surface	Smooth	Hairy	Smooth or warty	No spores	No spores
Acid-fast stain	±	−	−	±	+
Chemical characters					
Wall type	IV	IV	III	IV	IV
Nocardomycolic acid	+†	−	−	+	−
Mycolic acid *sensu stricto*	−	−	−	−	+
Hydrolysis tests					
Casein	±	+	+	−	−
Elastin	±	+	+	−	−
Sole carbon sources					
Cellobiose	−	+	−	−	−
Erythritol	−	+	−	−	NR
Lactose	−	+	−	−	−
α-Methyl-D-glucoside	−	+	−	−	NR
Raffinose	−	+	−	−	−
Sorbitol	−	+	−	+	±
Benzoate	−	+	−	±	±
Resistance to					
Lysozyme	+	−	−	−	−
Gentamycin	−	+	±	−	±
Rifampicin	+	+	+	−	+
Streptomycin	+	+	−	+	±

+, Character positive in more than 80 % of isolates; −, character negative in more than 80 % of isolates; ±, character variable, occurring in 21 to 79 % of isolates; NR, no result.

* Whether or not arthrospores are considered to occur in *Nocardia* depends on the interpretation placed on fragmenting aerial hyphae.

† *Nocardia autotrophica* not included.

differentiated from related taxa. Characters positive in more than 80 % of strains are designated +, and those positive in fewer than 20 % of strains are designated −. *Saccharopolyspora hirsuta* may be differentiated from all related taxa by their characteristic spores and the pattern of utilization of organic compounds as sole carbon sources; from *Nocardia* by their lack of nocardomycolic acids, susceptibility to lysozyme and resistance to gentamycin; from *Actinomadura* by wall composition; and from *Mycobacterium* and '*Mycobacterium*' *rhodochrous* by the presence of aerial mycelium and spores, lack of mycolic or nocardomycolic acids, and antibiotic resistance.

Distribution. Saccharopolyspora hirsuta was frequently isolated from bagasse samples, although usually in small numbers (Lacey, 1974), and was present in samples originating from Puerto Rico, Trinidad, Jamaica and India. It was accompanied by thermophilic fungi and other actinomycetes, indicating spontaneous heating of the bagasse during storage.

Cultures of the type strain are deposited with the National Collection of Industrial Bacteria (NCIB 11079), Centraalbureau voor Schimmelcultures (CBS 420.74), American Type Culture Collection (ATCC 27875), Northern Regional Research Laboratories, Peoria, Illinois (NRRL B-5792) and the Kaken Chemical Co (KCC A-0170).

DISCUSSION

Recently, many novel actinomycetes have been isolated from different habitats. Some fit in established genera (Lacey, 1971*b*; Nonomura, 1974) but the others have required new genera (Gledhill & Casida, 1969; Nonomura & Ohara, 1971*a*), increasing the number recognized from 35 (Williams, Davies & Cross, 1968) to about 60 (Krassilnikov, 1970; Cross & Goodfellow, 1973). A classification should be practical and new taxa should not be proposed until it is certain that they do not fit those already established. However, poor descriptions and lack of a generally accepted classification may make this difficult. The isolates from bagasse form a strikingly homogeneous group on morphological, biochemical, chemical, nutritional and antibiotic-sensitivity criteria, but cannot be identified unequivocally by using published dichotomous or tabular keys (Williams *et al.* 1968; Lechevalier & Lechevalier, 1970; Prauser, 1970; Goodfellow & Cross, 1974). In their nutrition and ability to degrade a range of compounds they are similar to *Nocardia*, but they lack nocardomycolic acids, are sensitive to lysozyme, resistant to more antibiotics (Goodfellow & Orchard, 1974) and have a distinctive morphology. The fine structure of their spores differs from that reported in *Nocardia* although that of their hyphae is similar. Isolates at present classified as *Nocardia autotrophica* (Takamiya & Tubaki) Hirsch differ from other Nocardia species in lacking nocardomycolic acids although they have a type IV wall. Isolates from bagasse differ from this taxon in their characteristic morphology, and their ability to degrade casein and grow on benzoate (Gordon *et al.* 1974).

Gordona, *Micropolyspora*, '*Mycobacterium*' *rhodochrous* and *Saccharomonospora* are also placed in the family Nocardiaceae (Nonomura & Ohara, 1971*b*; Cross & Goodfellow, 1973). The bagasse isolates differed from these in morphology and fine structure, although Micropolyspora species also produce substrate and aerial mycelia but with chains of up to only 20 spores (usually fewer) having smooth, finely toothed or warty surfaces and with prominently thickened end walls. Nocardomycolic acids have been found in *Micropolyspora brevicatena* but not in *Micro. angiospora*. It is not known whether they occur in other Micropolyspora species.

Bacterionema, *Mycobacterium*, and *Pseudonocardia* also have type IV walls, but the first two produce at most a transient substrate mycelium, no aerial mycelium or spores, and differ in many other respects from the bagasse organism. *Pseudonocardia* has a stable mycelium and distinctive morphology.

Saccharopolyspora hirsuta isolates resemble *Actinomadura*, especially *A. dassonvillei*, in morphology, fine structure, nutrition and hydrolytic abilities, but differ in wall composition. There was less affinity with *A. madurae* (Vincent) Lechevalier & Lechevalier and *A. pelletieri* (Laveran) Lechevalier & Lechevalier, which were only loosely associated with *A. dassonvillei* by numerical taxonomy (Goodfellow, 1971). We do not know enough of the new species described by Nonomura & Ohara (1971*c*) to assess their relationships with other *Actinomadura* species.

The isolates from bagasse have affinities with species of Nocardia and Actinomadura and their classification depends on the emphasis placed on the various groups of characters. Unless *Nocardia* and *Actinomadura* are reunited (Krassilnikov, 1970; Gordon *et al.* 1974) it does not seem advisable to include the bagasse isolates in either genus. We prefer the dangers of 'splitting' rather than accept the alternative of 'lumping' the species into a heterogeneous taxon, especially as *Nocardia* has only recently ceased to be a 'taxon of convenience' for difficult isolates (Cross & Goodfellow, 1973). Chemical criteria should not outweigh other taxonomic criteria although they have recently helped improve classification

(Lechevalier *et al.* 1973). These conclusions would make it inadvisable to include organisms with different types of wall in the same genus. The bagasse isolates form a homogeneous group distinguishable from other taxa by several unrelated criteria. Because *Saccharopolyspora* gen. nov. is aerobic, has fragmenting vegetative mycelium, aerial mycelium forming spores, and type IV walls, the genus should be classified in the family Nocardiaceae (Lechevalier & Lechevalier, 1970; Prauser, 1970; Cross & Goodfellow, 1973).

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