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Thermoactinomyces sacchari sp.nov., a Thermophilic Actinomycete Causing Bagassosis

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SUMMARY

A new species of thermophilic monosporic actinomycete, isolated from mouldy sugar cane bagasse, is described as *Thermoactinomyces sacchari* sp.nov. It is distinguished from *T. vulgaris* Tsiklinsky by short tufted aerial mycelia that rapidly autolyse and are not always seen, by bearing spores on short sporophores, by its appearance on different culture media and by serological differences. Both species have heat-resistant spores containing dipicolinic acid and with the structure of bacterial endospores. A sufferer from bagassosis who inhaled extracts of *T. sacchari* developed symptoms characteristic of the disease.

INTRODUCTION

Bagassosis is a respiratory disease caused by inhaling dust from mouldy, self-heated, crushed sugar cane (bagasse). It is a form of extrinsic allergic alveolitis (Pepys, 1969) and clinically it is similar to farmer's lung. The role of thermophilic actinomycetes in the aetiology of the disease was therefore strongly suspected. Inhalation of extracts of *Thermoactinomyces vulgaris* by an Englishman and Trinidadians suffering from bagassosis produced symptoms resembling those of the disease (Hargreave, Pepys & Holford-Strevens, 1968; Hearn & Holford-Strevens, 1968). Also Salvaggio, Arquembourg, Seabury & Buechner (1969) found precipitins in sera of patients with bagassosis, using extracts of an actinomycete identified as *Micromonospora vulgaris* (Seabury, Salvaggio, Buechner & Kundur, 1968). *Micromonospora vulgaris* is a synonym of *T. vulgaris*, but Seabury *et al.* (1968) identified their isolates with the genus *Micromonospora* partly from confusing results from analysing mycelial hydrolysates with published data on purified wall hydrolysates.

I have recovered only few *Thermoactinomyces vulgaris* from mouldy bagasse, but large numbers of an unusual thermophilic actinomycete with characters of the genus *Thermoactinomyces* Tsiklinsky, 1899. This differs from *T. vulgaris* and is thought to be a new species, for which I propose the name *T. sacchari*. The Englishman who reacted to inhaled extracts of *T. vulgaris* (Hargreave *et al.* 1968) also reacted to *T. sacchari*. I have examined cultures named *Micromonospora vulgaris* by Seabury *et al.* (A 827) and 'beige actinomycete' by Nicholson (1968) (A 449), and would identify both as *T. sacchari*. The evidence strongly suggests that *T. sacchari* is the principal cause of bagassosis.

Isolates of *Thermoactinomyces sacchari* from bagasse were compared with those of *T. vulgaris* and descriptions of other thermophilic actinomycetes; also extracts were inhaled by a person susceptible to bagassosis and tested immunologically against sera from other sufferers.

METHODS

Isolation. Samples of mouldy sugar cane bagasse were received from various places where sugar cane is grown (Lacey, 1969, 1970). Actinomycetes were isolated by the wind tunnel technique of Gregory & Lacey (1963) except that bagasse samples were enclosed in small muslin bags to retain the fine particles, before placing them in the rotating drum of the wind tunnel. The isolates grew poorly and produced few spores on the half strength nutrient agar (Corbaz, Gregory & Lacey, 1963) used for isolating other actinomycetes, so they were isolated and grown on yeast extract agar (Corbaz *et al.* 1963) containing 50 μg. cycloheximide/ml. and incubated at 55° or 60°.

The sources of the isolates used are listed in Table 1.

Table 1. *Origin of isolates studied*

Laboratory reference no.	Source of isolate
A449	Beige colony isolated by D. P. Nicholson, Dallas, Texas, U.S.A., from bagasse
A827	*Micromonospora vulgaris* isolated by Seabury *et al.*, New Orleans, Louisiana, U.S.A., from bagasse
A978–991	*Thermoactinomyces sacchari* isolated from bagasse from Trinidad
A998–999	*T. sacchari* isolated from bagasse, source unknown
A1000	*T. sacchari* isolated from bagasse from the West Indies
A1001–1002	*T. sacchari* isolated from bagasse from Puerto Rico
A1004–1008	*T. sacchari* isolated from bagasse from Mauritius
A64	*T. vulgaris* isolated from hay, Rothamsted
A351	*T. vulgaris* isolated from straw, from Newbury, Berkshire
A549	*T. vulgaris* isolated from air in a barley silo, Grantham, Lincolnshire

Media. Media used for characterization were: nutrient agar (Oxoid CM3); nutrient agar with glucose (Cross, Maciver & Lacey, 1968*a*); yeast extract agar (Corbaz *et al.* 1963); glucose yeast extract agar (Cross *et al.* 1968*a*); milk agar (Cross *et al.* 1968*a*); peptone yeast extract iron agar (Shirling & Gottlieb, 1966; potato carrot agar (Cross, Lechevalier & Lechevalier, 1963); potato glucose agar (Baldacci, Comaschi, Scotti & Spalla, 1953); chitin agar (Lingappa & Lockwood, 1962); carbohydrate utilization media (Shirling & Gottlieb, 1966), basal mineral salts agar with the addition alternatively of no carbohydrate source of 1 % (w/v) of D-glucose, L-arabinose, sucrose, D-xylose, I-inositol, D-mannitol, D-fructose, rhamnose, raffinose of cellulose; nutrient agar with carbohydrate sources added as above.

Colony morphology. Cultures were examined on the surface of agar plates using 40 × long working distance or conventional objectives. Substrate and aerial mycelium were classified as described by Cross *et al.* (1968*a*). Colour descriptions are those of Ridgway (1912). Spores were also examined by transmission electron microscopy in silhouette and after sectioning, and by a 'Stereoscan' electron microscope.

RESULTS

Thermoactinomyces sacchari isolates produced spores singly on both aerial and substrate mycelia, a characteristic of the genus *Thermoactinomyces*, but differed consistently in colony morphology and cultural characteristics from the *T. vulgaris* isolates. Both species grew rapidly at 55° on suitable media, but *T. sacchari* characteristically produced spores on sporophores and short tufted aerial mycelium (Pl. 1*b*), contrasting with the generally sessile

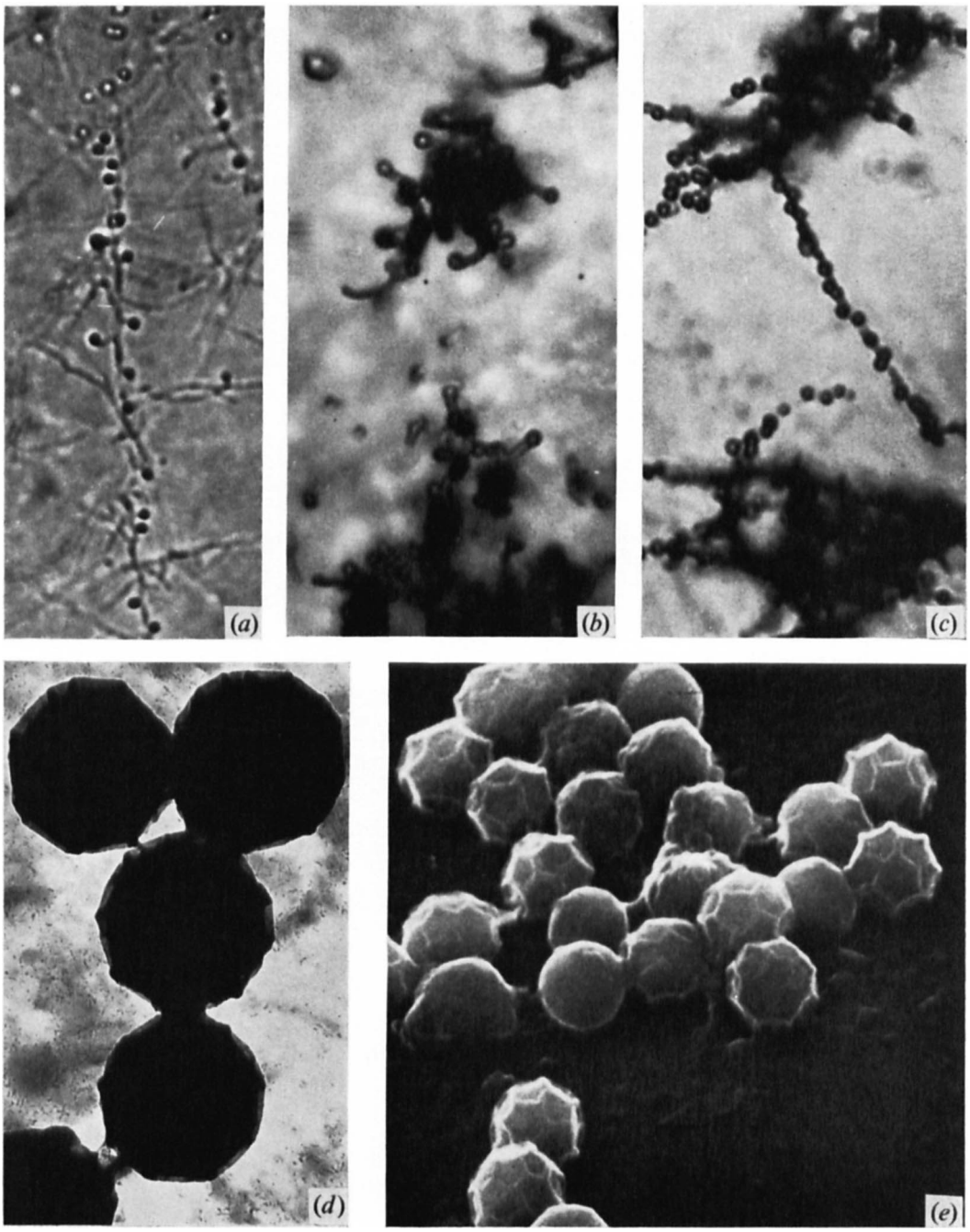

PLATE 1

(*a*) Substrate mycelium beneath agar surface. Nutrient agar. *Thermoactinomyces sacchari* A 449. × 1600.
(*b*) Aerial mycelium. Potato glucose agar. *T. sacchari* A 1004. × 1300.
(*c*) Aerial mycelium. Nutrient with glucose agar. *T. vulgaris* A 351. × 1300.
(*d*) Electron micrograph of aerial mycelium spores. *T. sacchari* A 989. × 28,500.
(*e*) 'Stereoscan' electron micrograph of aerial mycelium spores. *T. sacchari* A 978. × 10,000.

spores and long aerial hyphae of *T. vulgaris* (Pl. 1 *c*). Mycelium of *T. sacchari* soon lysed, leaving spores in a thick layer on the agar surface, and giving the colonies a bacterial appearance.

Appearance on various media. Growth of *Thermoactinomyces sacchari* and *T. vulgaris* at 55° was compared on a range of media. Both species seldom grew well on the same medium and few media permitted growth of aerial mycelium of *T. sacchari*. *Thermoactinomyces sacchari* grew best, and produced aerial mycelium on many colonies, on nutrient agar with glucose, yeast extract agar, glucose yeast agar, potato carrot agar and potato glucose agar. *Thermoactinomyces vulgaris* produced aerial mycelium on most media tested, but grew best

Table 2. *Appearance of Thermoactinomyces sacchari and T. vulgaris on different media*

Medium	*Thermoactinomyces sacchari*	*Thermoactinomyces vulgaris*
Nutrient agar	G. poor, thin, almost transparent, few spores	G. good, flat except for slight ridging at centre, abundant sporulation
	A.M. none	A.M. white but A 549 becoming pale cinnamon pink–tilleul buff
	Rev. colourless—pale olive buff	Rev. deep olive buff at centre grading to white at edge A 549 near walnut brown
Nutrient agar with glucose	G. moderate, colonies ridged, becoming olive buff with autolysis of A.M., abundant sporulation	G. very good, abundant sporulation
	A.M. white, variable, sparse, limited to sectors or over most of colony. Less after 5 days	A.M. white (tilleul buff in A 549)
	Rev. colourless or modified by pale yellow brown S.P.	Rev. tawny olive at centre with darker mottling grading to colourless at edge
Yeast extract agar	G. good, colonies ridged, slimy appearance, good abundant sporulation	G. poor, restricted, irregularly furrowed, little sporulation
	Where no A.M. but thick layer of spores on agar surface, olive buff; when few spores, olive brown	A.M. sparse, white
	A.M. white, transient, on fewer than half of the colonies. Less after 5 days	Rev. colourless
	Rev. colourless—olive buff—olive brown.	

Key to abbreviations: G. = growth; A.M. = aerial mycelium; Rev. = reverse; S.P. = soluble pigment
Except where stated, descriptions refer to colonies after 3 days incubation at 55°.

Table 3. *Carbohydrate assimilation by Thermoactinomyces sacchari and T. vulgaris*

Carbohydrate	*Thermoactinomyces sacchari*	*Thermoactinomyces vulgaris*
Glucose	++	++
Arabinose	+	−
Cellulose	−	−
Fructose	++	++
Inositol	−	−
Mannitol	++	++
Raffinose	−	−
Rhamnose	−	−
Sucrose	−	+
Xylose	−	−

Carbohydrate sources were incorporated in nutrient agar to 1 % (w/v), growth similar to that on unamended control medium; +, more growth than on unamended control medium but less than on glucose amended medium; ++, growth similar to that on glucose amended medium.

on nutrient agar with glucose. Table 2 compares and contrasts the growth of *T. sacchari* and *T. vulgaris* on three of the media tested. Yeast extract agar allowed only *T. sacchari* to grow well, and nutrient agar only *T. vulgaris*. Although both species grew well on nutrient agar with glucose, they differed in colony appearance and morphology.

Both *Thermoactinomyces sacchari* and *T. vulgaris* rapidly digested casein when grown on milk agar and cleared chitin agar, although growth was poor. Neither produced melanoid pigments in peptone iron agar.

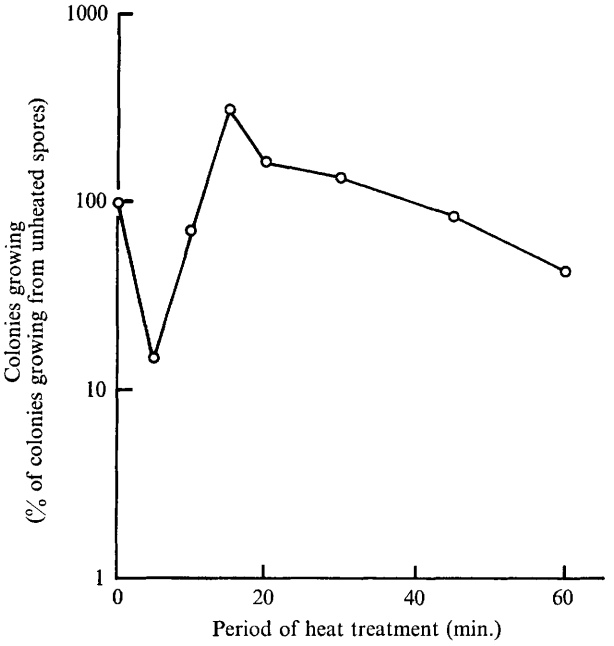

Fig. 1. Heat activation and resistance of *Thermoactinomyces sacchari* spores at 100°.

Carbohydrate assimilation. Thermoactinomyces sacchari grew poorly on basal mineral salts agar containing all the carbohydrate sources tested. Only glucose gave a slight increase in growth. *Thermoactinomyces sacchari* also grew poorly on nutrient agar, but showed a large growth response to the addition of some carbohydrate sources (Table 3). Although *Thermoactinomyces vulgaris* grew better on nutrient agar than *T. sacchari*, responses to added carbohydrate sources could also be observed (Table 3).

Novobiocin resistance. The growth of *Thermoactinomyces sacchari* was unaffected by adding 25 μg. novobiocin/ml. half-strength nutrient or yeast extract agars. Even 100 μg./ml. had little effect on the scant growth on half-strength nutrient agar, but decreased growth on yeast extract agar. *Thermoactinomyces vulgaris* grew well on half-strength nutrient agar containing 100 μg. novobiocin/ml., but not at all on yeast extract agar containing only 25 μg./ml.

Ability to grow at different temperatures. Both *Thermoactinomyces sacchari* and *T. vulgaris* grew on yeast extract or nutrient agars in Petri dishes between 35° and 65°. They did not grow at 33° and 67°. Growth was optimal at 55° to 60°.

Resistance to high temperatures. Washed spores of *Thermoactinomyces sacchari*, harvested from yeast extract agar incubated for 3 days at 55°, were suspended in M-1000 phosphate

buffer (pH 7·0) and sealed in glass ampoules. Survival curves obtained after holding the ampoules in mineral oil at 100° (Cross, Walker & Gould, 1968*b*) gave a $D_{100}°$ value of 59 min. (Fig. 1). The survival curves indicated that spores were activated by heat, and germination was greatest after 15 min. exposure. At first the temperature of the oil bath was depressed to about 90°, and until it reached near 100° germination was less than that of unheated spores, suggesting that more became dormant during the short period of sub-optimal heating.

Stimulation of spore germination. Growth of visible colonies on yeast extract agar was used to measure if germination of *Thermoactinomyces sacchari* spores was stimulated by chemicals (Hills, 1949; Riemann & Ordal, 1961). Spores were either (*a*) incubated at 55° for 30 min. before plating, in suspensions containing calcium chloride and sodium dipicolinate in concentrations sufficient to give 40 mM of the 1:1 Ca-DPA chelate, or containing 1 or 10 mM L-alanine or unamended; or (*b*) plated onto agar containing 1 or 10 mM L-alanine or unamended.

Table 4. *Growth of Thermoactinomyces sacchari colonies after chemical treatment of the spores*

Chemical ...	L-Alanine		Calcium dipicolinate
Concentration ...	1 mM	10 mM	40 mM
	No. colonies growing, as % of control		
Chemical added to spore suspension*	171	156	140
Chemical added to yeast extract agar	105	116	—

* Spore suspension incubated 30 min. at 55° before diluting and plating on yeast extract agar.

All treatments increased the number of colonies visible after 24 h. at 55°, compared with the unamended controls (Table 4), but adding L-alanine to agar was less effective than adding the same concentrations to the spore suspension before plating.

Analysis of mycelial hydrolysates and spore contents. Mycelial hydrolysates of *Thermoactinomyces sacchari*, prepared and analysed as described by Lechevalier (1968), contained only the *meso*-isomer of diaminopimelic acid, ribose and a trace of galactose consistent with a 'type c' sugar pattern. Diaminopimelic acid was less easy to detect in 3 day autolysed cultures than in younger cultures not yet autolysed.

Spores of *Thermoactinomyces sacchari* analysed as described by Lewis (1967) contained 7 % dipicolinic acid, a similar amount to that found by Cross *et al.* (1968*b*) in spores of *T. vulgaris*.

Occurrence. Thermoactinomyces sacchari was isolated from the surface of freshly harvested sugar cane, and from muds from filter presses at the sugar mills, but was most abundant in mouldy self-heated bagasse. Freshly baled bagasse contains 50 % water and about 3 % sugar, and heats rapidly after stacking. The temperature may remain above 40° for several weeks, with a maximum of 50° to 60°, providing good growing conditions for thermophilic actinomycetes.

Pathogenicity. There is no evidence that *Thermoactinomyces sacchari* can grow in the lungs, but inhalation of aerosols containing extracts of *T. sacchari* have produced symptoms typical of bagassosis in a patient. Professor J. Pepys, Institute of Diseases of the Chest, Brompton, London S.W. 3, has tested a patient, who developed bagassosis in England, with aerosols containing extracts of cultures of A 827 and A 970 grown on yeast extract agar

and prepared as described by Pepys *et al.* (1963). Inhalation of 0·9 ml. of reconstituted extract of A 979 containing 1 mg. extract/ml. caused a systemic febrile reaction, which, starting after 11 h. was greatest after 18 h. and disappeared only after 24 h. This was accompanied by aching, tightness of the chest, burning sensation in the eyes, headache, sweating and an increase in white blood cells.

The reaction was faster in a later test with a dose of 0·7 ml. of reconstituted extract of A 827, containing 10 mg. extract/ml. Generalized aches and pains developed after 5 h. and a febrile reaction started after 6 h., was most pronounced after 10 h., and disappeared after 21 h. Symptoms were otherwise as in the previous test, and a decrease in the CO gas transfer factor was noted.

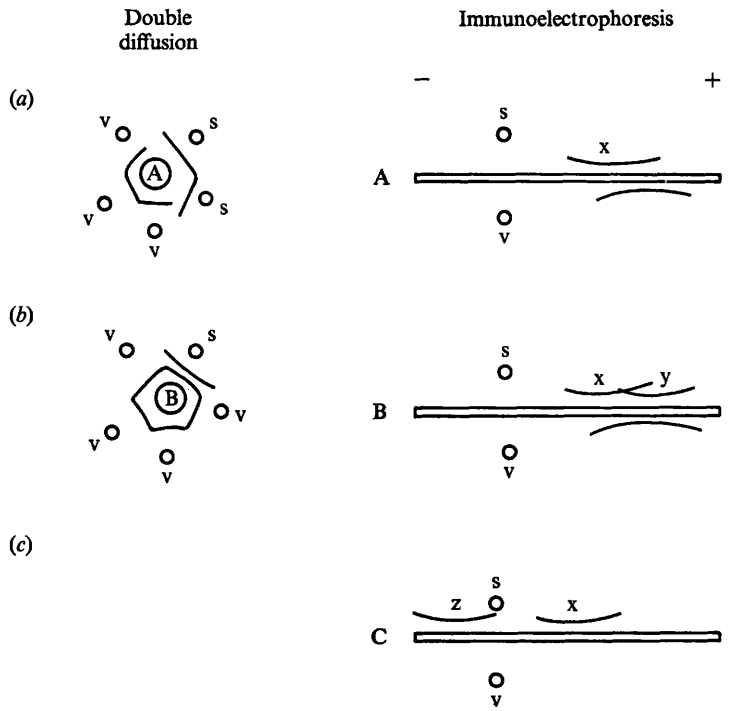

Fig. 2. Precipitin reactions in double diffusion and immunoelectrophoretic tests of extracts of *Thermoactinomyces sacchari* (s) and *Thermoactinomyces vulgaris* (v) with sera from bagassosis patients (A, B, C).

Characteristic precipitin reactions were obtained when extracts of *Thermoactinomyces sacchari* were tested by double diffusion and immunoelectrophoresis against sera from bagassosis patients in Dallas, Texas, U.S.A., and from workers exposed to bagasse dust in Trinidad (Fig. 2, x arc) (V. Holford-Strevens, personal communication). These could not be removed by adsorption of the sera with extracts of *T. vulgaris*. *Thermoactinomyces vulgaris* extracts occasionally reacted with sera from bagassosis patients (Fig. 2), but precipitin arcs were formed in a different region from those of *T. sacchari* indicating that the antigens were differently charged. Occasionally *T. sacchari* extracts produced other precipitin arcs, one in the same region as *T. vulgaris* (Fig. 2*b*, x arc), suggesting that there may be common antigens in the two species, and another on the cathodic side of the antigen well (Fig. 2*c*, z arc). Reactions were obtained with extracts of cultures grown at 37° to 60°

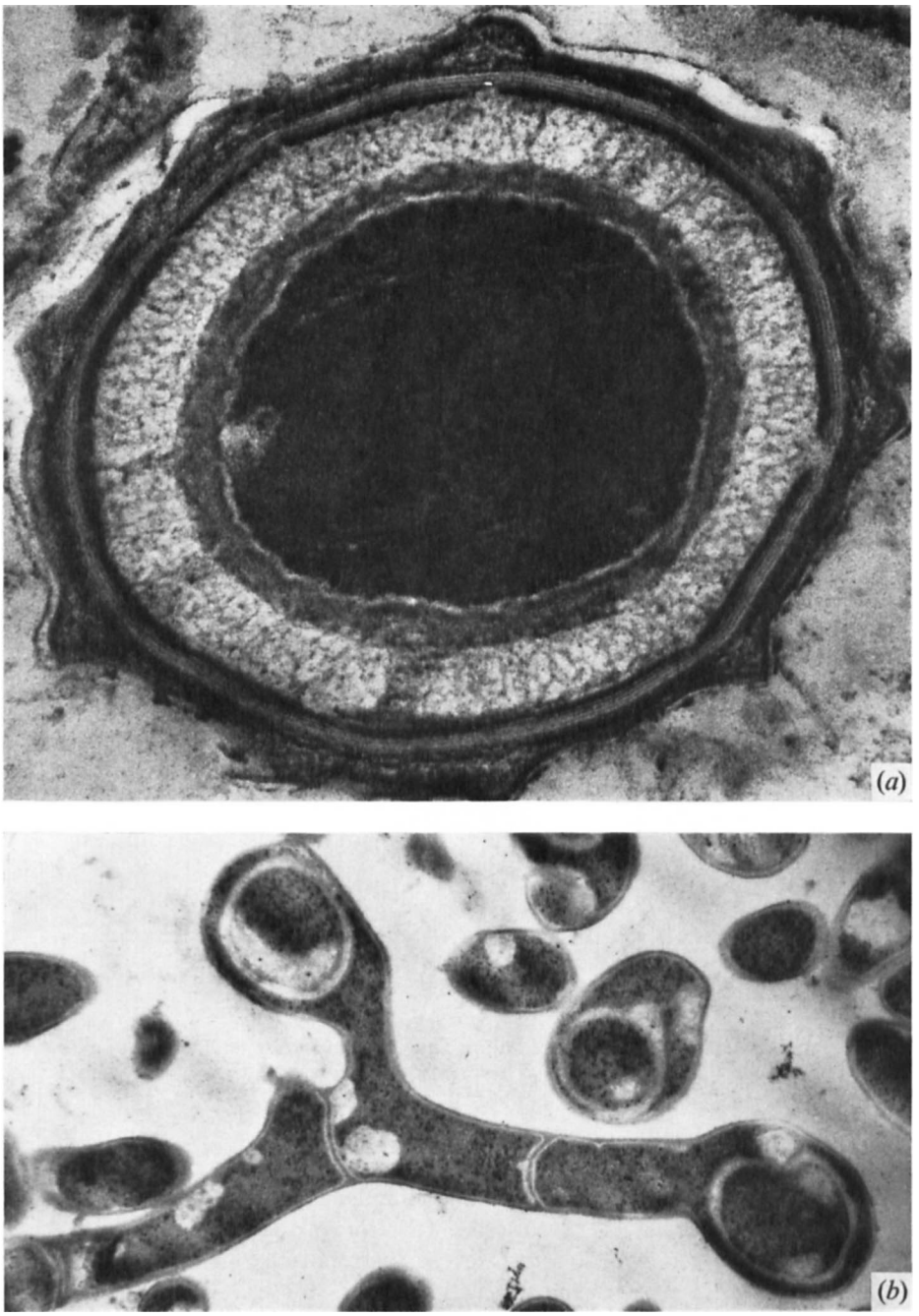

PLATE 2

(*a*) Electron micrograph of sectioned spore of *Thermoactinomyces sacchari* A978. × 111,000.

(*b*) Electron micrograph of sectioned hypha of *T. sacchari* A978 showing sporophore formation and forespores. × 30,000.

on yeast extract and half-strength nutrient agars, and at 55° on potato glucose, nutrient, nutrient with glucose, Lab-Lemco, glucose yeast, potato–carrot and V 8 agars, but not with cultures grown on inorganic salts starch agar. The reactivity of the extracts depended on the amount of growth in culture. The strongest reactions were obtained with extracts from cultures grown on yeast extract agar incubated between 40° and 60° and on nutrient with glucose agar grown at 55°.

Description of **Thermoactinomyces sacchari** Lacey sp.nov. (sacchari = of sugar cane) Type strain A 978.

Substrate mycelium. About 0·6 to 0·8 μm. diameter, septate (Pl. 2*b*). Hyphae branching, penetrating the agar medium forming fast-growing, spreading colonies, colourless at first, becoming cartridge buff with many spores. Colonies ridged irregularly, becoming slimy and bacterial in appearance as hyphae autolyse. Spores formed singly, usually on short sporophores within the agar and on the surface (Pl. 1*a*). Sporophores formed as outgrowths of hyphal cells after the initiation of spore development (Lacey & Vince, 1971).

Table 5. *Comparison of Thermoactinomyces sacchari and T. vulgaris*

Character	*Thermoactinomyces sacchari*	*Thermoactinomyces vulgaris*
Similarities:		
Single spores on aerial mycelium	+	+
Single spores on substrate mycelium	+	+
Spores endospores with ridged surface	+	+
Spores containing dipicolinic acid	+	+
Spores heat-resistant	+	+
Spores activated by heat	+	+
Aerial mycelium colour	White	White
Mycelial walls containing *meso*-diaminopimelic acid	+	+
Cell sugar pattern (Lechevalier, 1968)	Type c	Type c
Mycelium septate	+	+
Differences:		
Spores on sporophores	Mostly; up to 3 μm. long	Rarely; up to 1 μm.
Spores sessile	Rarely	Mostly
Aerial hyphae	Sparse, short, tufted	Abundant, long, arching
Lysis of aerial mycelium	Rapid, within 3 days	Not seen
Substrate mycelium colour	Colourless to cartridge buff	Colourless to brown
Soluble pigment	Yellow-brown (some isolates and media only)	Not seen
Assimilation of sucrose	−	+
Assimilation of arabinose	+	−
Growth on nutrient agar	Poor, thin, no aerial mycelium	Good, spreading, abundant aerial mycelium
Growth on yeast extract agar	Good, spreading, usually with some aerial mycelium	Poor, restricted, little aerial mycelium
Precipitin reactions with bagassosis sera on immunoelectrophoresis	x arc charateristic, sometimes y and z arcs also	Sometimes; y arc only

Aerial mycelium. About 0·8 to 1·0 μm. diameter, septate, white, often limited to irregular areas or sectors of the colony, sometimes none. Transient, usually disappearing within 2 to 3 days through autolysis of the hyphae. Hyphae short, tufted, with spores laterally usually on short sporophores up to 3 μm. long, and terminally (Pl. 1*b*), sometimes appearing dichotomously branched, but electron microscopy shows that this is due to outgrowth of adjacent cells during sporulation, and not true dichotomy (Pl. 2*b*).

Spores. Refractile, appearing globose with the light microscope, but angular in transmission electron micrographs because of a pattern of ridges on the spore surface (Pl. 1 *d*, *e*). Spores with a structure resembling bacterial endospores (Pl. 2 *a*) and showing similar stages of development (Lacey & Vince, 1971). Heat-resistant, containing dipicolinic acid, and staining with stains specific for bacterial endospores.

The type strain is deposited at the Centraalbureau voor Schimmelcultures, Baarn, Netherlands (CBS 701.70), the National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London N.W. 9 (NCTC 10721), and the National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, Scotland (NCIB 10486).

Table 5 compares features of *Thermoactinomyces sacchari* and *T. vulgaris*. The similarities between the two species are mostly characteristics of the genus *Thermoactinomyces*. Septa were seen in longitudinal sections of hyphae of both *T. sacchari* and *T. vulgaris*, although the latter has previously been described as having non-septate hyphae (Waksman 1961). Other characters differentiate the two species. Although most spores of *T. sacchari* were on sporophores up to 3 μm. long, occasionally some spores were sessile. No sporophores were seen in cultures of *T. vulgaris*. These have been reported but are usually less than about 1 μm. long (Waksman, Umbreit & Cordon, 1939).

DISCUSSION

Thermoactinomyces sacchari is the third actinomycete to be implicated in forms of extrinsic allergic alveolitis. *Micropolyspora faeni* and *T. vulgaris* have both been implicated in farmer's lung (Pepys *et al.* 1963), some forms of fog fever of cattle (Jenkins & Pepys, 1965) and possibly also in mushroom workers' lung (Sakula, 1967). Other actinomycetes may also cause similar diseases, so incubation temperatures that favour thermophiles should be used when seeking possible causes.

The properties and structure of the spores of *Thermoactinomyces sacchari* resemble spore-forming bacteria of the genera *Bacillus* and *Clostridium*. Two other actinomycetes, *T. vulgaris* and *Actinobifida dichotomica*, have similar spore structure (Cross *et al.* 1968*b*). These species share many characters and their separation into different genera is questionable. The genus *Actinobifida* was created for species with dichotomously branched sporophores (Krassilnikov & Agre, 1964), although dichotomous branching was already accepted in *Micromonospora* and *Thermomonospora*. *Thermoactinomyces sacchari* with spores on short sporophores provides a morphology intermediate between the sessile spores of *T. vulgaris* and the long, dichotomously branched sporophores of *A. dichotomica*. Spore structure should probably take priority over sporophore morphology in determining generic disposition. Cross & Lacey (1970) suggested that Actinobifida species with spores like bacterial endospores should be placed in *Thermoactinomyces*, and others with spores resembling fungal aleuriospores (Cross, 1970) in *Thermomonospora*. The intermediate position of *T. sacchari* gives further support to this suggested reclassification.

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REFERENCES

BALDACCI, E., COMASCHI, G. F., SCOTTI, T. & SPALLA, C. (1953). General criteria for the systematics of genera and species of *Actinomyces* (*Streptomyces*) and *Micromonospora*. *VIth International Congress of Microbiology. Symposium: Actinomycetales.* (*Supplemento ai reconditi del L'istituto superiore di sanitá*), pp. 20–39.

CORBAZ, R., GREGORY, P. H. & LACEY, M. E. (1963). Thermophilic and mesophilic actinomycetes in mouldy hay. *Journal of General Microbiology* **32**, 449–455.

CROSS, T. (1970). The diversity of bacterial spores. *Journal of Applied Bacteriology* **33**, 95–102.

CROSS, T., LECHEVALIER, M. P. & LECHEVALIER, H. (1963). A new genus of the Actinomycetales: *Micro-ellobosporia* gen.nov. *Journal of General Microbiology* **31**, 421–429.

CROSS, T. & LACEY, J. (1970). Studies on the genus *Thermomonospora*. In *The Actinomycetales*, pp. 211–219. Edited by H. Prauser. Jena: Gustav Fischer.

CROSS, T., MACIVER, A. M. & LACEY, J. (1968*a*). The thermophilic actinomycetes in mouldy hay: *Micro-polyspora faeni* sp.nov. *Journal of General Microbiology* **50**, 351–359.

CROSS, T., WALKER, P. D. & Gould, G. W. (1968*b*). Thermophilic actinomycetes producing resistant endo-spores. *Nature, London* **220**, 352–354.

GREGORY, P. H. & LACEY, M. E. (1963). Mycological examination of dust from mouldy hay associated with farmer's lung disease. *Journal of General Microbiology* **30**, 75–88.

HARGREAVE, F. E., PEPYS, J. & HOLFORD-STREVENS, V. (1968). Bagassosis. *Lancet* i, 619–620.

HEARN, C. E. D. & HOLFORD-STREVENS, V. (1968). Immunological aspects of bagassosis. *British Journal of Industrial Medicine* **25**, 283–292.

HILLS, G. M. (1949). Chemical factors in the germination of spore-bearing aerobes. The effect of amino acids in the germination of *Bacillus anthracis* including observations on the specificity of some stereo-isomers. *Biochemical Journal* **45**, 363–370.

JENKINS, P. A. & PEPYS, J. (1965). Fog-fever. Precipitin (FLH) reactions to mouldy hay. *Veterinary Record* **77**, 464–466.

KRASSILNIKOV, N. A. & AGRE, N. S. (1964). A new actinomycete genus—*Actinobifida* n.gen. The yellow group—*Actinobifida dichotomica* n.sp. *Mikrobiologiya* **33**, 935–943.

LACEY, J. (1969). Bagassosis. *Rothamsted Experimental Station Report for* 1968 part 1, p. 133.

LACEY, J. (1970). Bagassosis. *Rothamsted Experimental Station Report for* 1969 part 1, p. 173.

LACEY, J. & VINCE, D. A. (1971). Endospore formation and germination in a new Thermoactinomyces species. In *Spore Research*—1971. Edited by A. N. Barker, G. W. Gould & J. Wolf. London: Academic Press (in the press).

LECHAVALIER, M. P. (1968). Identification of aerobic actinomycetes of clinical importance. *Journal of Laboratory and Clinical Medicine* 71, 934–944.

LEWIS, J. C. (1967). Determination of dipicolinic acid in bacterial spores by u.v. spectrophotometry of the calcium chelate. *Analytical Biochemistry* **19**, 327–337.

LINGAPPA, Y. & LOCKWOOD, J. L. (1962). Chitin media for selective isolation and culture of actinomycetes. *Phytopathology* **52**, 317–323.

NICHOLSON, D. P. (1968). Bagasse worker's lung. *American Review of Respiratory Diseases* **97**, 546–560.

PEPYS, J. (1969). *Hypersensitivity diseases of the lungs due to fungi and organic dusts. Monographs in Allergy*, vol. 4. Basle: S. Karger.

PEPYS, J., JENKINS, P. A., FESTENSTEIN, G. N., GREGORY, P. H., LACEY, M. E. & SKINNER, F. A. (1963). Farmer's lung: thermophilic actinomycetes as a source of 'farmer's lung hay' antigen. *Lancet* ii, 607–611.

RIDGWAY, R. (1912). *Colour Standards and Colour Nomenclature*. Washington: R. Ridgway.

RIEMANN, H. & ORDAL, Z. J. (1961). Germination of bacterial endospores with calcium and dipicolinic acid. *Science, New York* **133**, 1703–1704.

SAKULA, A. (1967). Mushroom worker's lung. *British Medical Journal* iii, 708–710.

SALVAGGIO, J., ARQUEMBOURG, P., SEABURY, J. & BUECHNER, H. (1969). Bagassosis. IV. Precipitins against extracts of thermophilic actinomycetes in patients with bagassosis. *American Journal of Medicine* **46**, 538–544.

SEABURY, J., SALVAGGIO, J., BUECHNER, H. & KUNDUR, V. G. (1968). Bagassosis. III. Isolation of thermo-philic and mesophilic actinomycetes and fungi from mouldy bagasse. *Proceedings of the Society for Experimental Biology and Medicine* **129**, 351–360.

SHIRLING, E. B. & GOTTLIEB, D. (1966). Methods for characterization of Streptomyces species. *International Journal of Systematic Bacteriology* **16**, 313–340.

TSIKLINSKY, P. (1899). Sur les mucedinées thermophiles. *Annales de l'Institute Pasteur, Paris* **13**, 500–505.

WAKSMAN, S. A. (1961). *The Actinomycetes*, vol. 2. London: Baillière, Tindall & Cox.

WAKSMAN, S. A., UMBREIT, W. W. & CORDON, T. C. (1939). Thermophilic actinomycetes and fungi in soils and in composts. *Soil Science* **47**, 37–61.