19. "Connective Tissue, Intracellular Macromolecules." Proc. Symp. New York Heart Assn. Little, Brown, Boston, Massachusetts (1964).

20. Hovig, T., Thromb. Diath. Haemorrhag. 9, 264 (1963).

Received May 7, 1968. P.S.E.B.M., 1968, Vol. 129.

Bagassois III. Isolation of Thermophilic and Mesophilic Actinomycetes and Fungi from Moldy Bagasse* (33319)

J. SEABURY, J. SALVAGGIO, H. BUECHNER, AND V. G. KUNDUR

Departments of Medicine and Microbiology (Clinical Immunology), Louisiana State University School of Medicine, New Orleans, Louisiana 70112, and the Medical Service, Veterans Administration Hospital, New Orleans, Louisiana 70112

Dried sugar cane fiber from which the liquid sucrose content has been extracted is known as bagasse. This material is commonly baled and allowed to remain exposed in the field for months before being processed into wall board and paper products. During storage it is subject to high environmental temperature and humidity and serves as a growth medium for myriads of soil fungi and bacteria. Approximately 5×10^8 fungal spores/g of dry weight have been estimated in the exposed specimens (1).

Some industrial workers who crush and grind the dry, raw material develop a characteristic respiratory illness called bagassosis that resembles other forms of "extrinsic allergic alveolitis" (2) or "hypersensitivity pneumonitis" (3). These include farmer's lung, sequoaosis, maple bark stripper's, mushroom picker's, thatched roof, pigeon breeder's, and pituitary snuff inhaler's diseases among others (4–12). Individuals with bagassosis demonstrate precipitating antibody against extracts of exposed moldy sugar cane fiber, suggesting that certain microorganisms growing in bagasse at high temperatures are involved in the etiology and pathogenesis of the disorder.

This report describes the most common thermophilic species isolated from bagasse samples obtained in Louisiana, Puerto Rico, and India.

* This study was supported by research grant AI 05754, U.S. Public Health Service, Bethesda, Maryland and the Louisiana. Tuberculosis and Respiratory Disease Association.

Materials and Methods. Organisms were isolated by grinding dry bagasse fiber in a Waring blendor for 10 min followed by exposure of sterile gelatin-coated Whatman No. 1 filter paper strips in the blendor flask atmosphere at 5, 10, 15, and 20-min intervals. Strips were immediately transferred by means of a sterile forceps into Petri dishes containing several media.

The media used included half-strength nutrient agar, yeast extract agar, V_8 agar, potato agar, and peptone iron agar. Petri dishes were incubated at 45°, and 0.5 mg of actidione/ml was added to inhibit fungal growth. Half-strength nutrient agar and yeast extract agar generally yielded the highest numbers of thermophilic microorganisms; peptone iron agar was used only for the melanin test.

Amino acid and sugar compostion of selected actinomycete whole-cell hydrolysate preparations were determined by descending paper chromatography on Whatman No. 1 filter paper by a modification of the technique of Becker, Lechevalier, and Lechevalier (13). Organisms from which cell-wall preparations were made were harvested from yeast extract or half-strength nutrient broth after incubation periods varying between 3 and 5 days. Broth was centrifuged at 1500 rpm then washed directly with sterile distilled water and ground for 15–20 min under sterile conditions using a Kontes glass grinder in an ice bath. Ground cells were immediately lyophilized. Hydrolysis and chromatography

TABLE I. Characteristics of Common Thermophilic Actinomycetes and Fungi Isolated from Bagasse.

Organism	Colonial morphology	Microscopic morphology	Culture medium	Electron micrographic spore morphology
Micromonospora vulgaris	Yellow-tan translucent wrinkled surface	Aerial mycelium short; single globose spores; abundant vegetative mycelium; hyphae 1 μ diameter	Half-strength nutrient; yeast extract	Round to globose 0.5–1.0 μ
Streptomyces thermoviolaceus	Variable; white becoming brown to ash gray or lavender; deep-maroon pigment with aging	Aerial hyphae short, flexuous; sporophores 40–80 μ with vegetative mycelium abundant	Half-strength nutrient; yeast extract	Barrel-shaped 0.8–1.2 $\times$ 0.6–1.0 μ
Streptomyces griscoflavus	Velvety; ash gray to white; reverse of colonies yellowish brown	Aerial hyphae show monopodial branching with spore chains in loose spirals	Half-strength nutrient	Oval to rectangular 0.8–1.0 $\times$ 0.4–0.8 μ nonuniform spiney projections
Streptomyces fradiae	Light pink to grayish pink; reverse of colonies yellowish brown	Aerial mycelium monopodial branching with loose and regular spirals	Yeast extract	Ovoid to oblong 0.8–1.2 $\times$ 0.5–0.8 μ smooth with slight surface irregularities
Streptomyces thermovulgaris	Lavender brown with white growing margin	Aerial mycelium abundant; some loose spirals with monopodial branching, but primarily regular tight spirals	Half-strength nutrient	Ovoid to oblong 1.0–1.2 $\times$ 1.4–1.6 μ smooth
Streptomyces olivaceus	Ash gray or lavender to bone white—often mixed—rugose to velvety	Aerial mycelium abundant and short. Hyphae straight or flexuous with monopodial branching	Yeast extract	Smooth to oval to cylindrical 0.9–1.0 $\times$ 0.4–0.8 μ
Humicola lanuginosa	White, cottony, felt-like, becoming grayish brown with aging	Aerial mycelium abundant, aleuriophores at right angles to hyphae	Yeast extract	Single aleuriospores 6–12 μ diameter

were then performed as described by Becker *et al.* (13–15).

Results. Many thermophilic bacteria, actinomycetes, and fungi were grown from all bagasse samples. Table I shows the result. Actinomycetes and fungi repeatedly isolated from a broad range of bagasse samples were, however, limited to a few species. Since current classification of thermophilic actinomycetes is controversial and ability to grow at various temperatures may be a function of nutritional requirements rather than a basic characteristic of a microorganism, we have tried to follow the classification of Ettlinger (16) for streptomycetes, which is based primarily on morphology. Thermophilic fungi were identified according to Cooney and Emerson (17). No attempt was made to identify thermophilic bateria isolated from bagasse or to isolate and identify fungi culturable from bagasse at temperatures other than the range of 45–60°. Neither was it determined whether isolates were truly thermophilic or merely thermotolerant.

Thermophilic microorganisms repeatedly isolated from bagasse specimens, including five exposed samples from Southern Louisiana obtained at sugar-cane processing plants, three from Puerto Rico, and one from Uttar Pradish State, India, are described as follows:

Our most frequent isolate was *Micromonospora vulgaris* Ørskov 1923 (18). This taxon is selected because our isolates had a type II cell wall (glycine, mesodiaminopimelic acid, alanine, glutamic acid, muramic acid, and glucosamine) and despite the fact that all showed conidia on both aerial and substrate mycelium. If morphology alone were used as the criterion, these isolated might be classified as *Thermoactinomyces vulgaris* Tsiklinsky 1899 (25).

All isolates were uniform in colonial and microscopic morphology. Growth and colonial characteristics were similar on both half-strength nutrient and yeast extract agar. Colonies appeared within 24 hr of incubation between 45 and 60°. Optimum temperature for growth was 54–56°.

Single colonies were almost always between 2 and 5 mm in diameter, thin, paler than yellow, or translucent white when viewed from the reverse (especially on yeast extract agar). Wrinkling of the colony surface was delicate, with peripheral crimping and radii from a small central, slightly elevated "button" in older colonies. Aerial mycelium of short, colorless-to-white type appeared early and peripherally, but disappeared after 48 to 72 hr of continued incubation (45–60°). There was no reaction on peptone iron agar.

Slide cultures (Fig. 1j) revealed most of the mycelium to be submerged (vegetative; primary) with hyphae 1 μ or less in diameter. Both aerial and submerged hyphae bore single, round-to-globose spores, 0.5–1.0 μ in diameter. These conidia were sessile for the most part, but occasionally short sporophores could be seen.

Electron micrographs (Fig. 2d and e) revealed the spore morphology to be apparently identical with that of the organism isolated from moldy hay by Corbaz, Gregory, and Lacey (1963) and identified by them as *M. vulgaris* (19). Although our organisms bore spores on both aerial and submerged mycelium as is noted in *T. vulgaris* (20), paper chromatography of cell hydrolysates revealed alanine, glucosamine, glutamic acid, and muramic acid plus glycine and meso-aE-diaminopimelic acid. These findings place our isolates in type II "Micromonospora type" of Becker, Lechevalier, and Lechevalier, 965 (13). One isolate that has given multiple precipitin arcs in double-diffusion analysis with several human antibagasse sera also contained galactose as a cell-wall constituent. *T. vulgaris* strains of Becker, Lechevalier, and Lechevalier all possessed a "madurae type" (type III) cell wall that did not contain glycine (13).

Our second most frequent isolate was *Streptomyces thermoviolaceus* Henssen, 1957 (20). Whether grown on half-strength nutrient or yeast extract agar, this organism showed much greater variation in gross colonial characteristics than did *M. vulgaris*. Growth was more rapid on yeast extract agar and supported more aerial mycelium. Colonial growth appeared within 3 or 4 days, but then spread slowly with a predominantly submerged mycelium. Young colonies usually

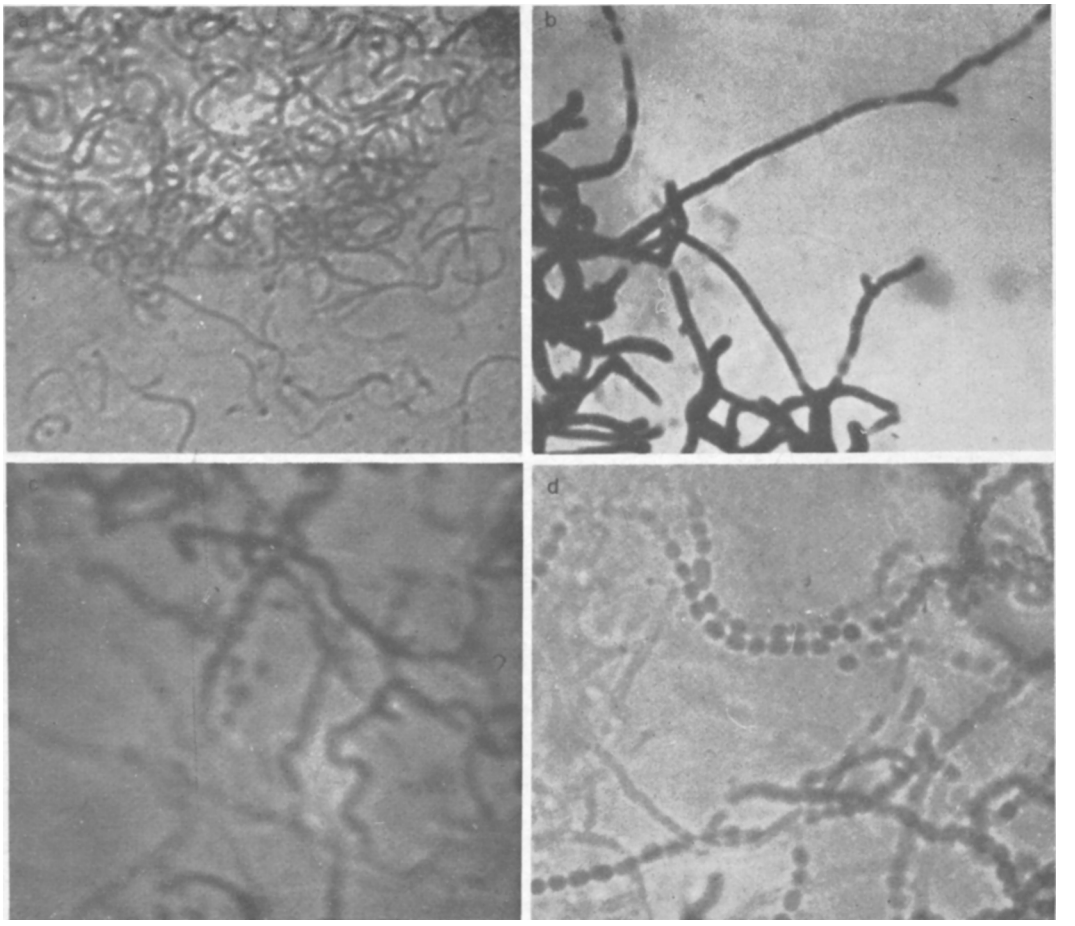

FIG. 1. (a) *S. thermoviolaceus*, slide culture-loose spirals; (b) *Pseudonocardia*, slide culture; (c) *S. griseoflavus*, slide culture-loose spirals; (d) *S. fradiae* spore chains, slide culture-loose spirals; (e) *S. thermoviolaceus*, spore chains; slide culture; (f) *Thermopolyspora*, Corbaz, Gregory, and Lacey (19), slide culture; (g) *S. thermovulgaris*, slide culture-tight spirals; (h) *S. griseoflavus*, slide culture-loose and tight spirals; (i) *Humicola lanuginosa*, slide culture; (j) *M. vulgaris*, slide culture.

showed a small button of aerial mycelium centrally. Growth was better and pigment changes more predicatable at 45° than at 60°.

The aerial hyphae were relatively short, but longer centrally than peripherally in slide cultures. The sporophores were of variable length, 40–80 μ on yeast extract agar, and were flexuous with approximately 25% showing loose spirals (Fig. 1a). Spore morphology was the most constant criterion for classification in this series. All strains showed barrel-shaped spores 0.8–1.2 $\times$ 0.6–1.0 μ covered with small wart-like oval projections (Fig. 2a).

Colonial growth was initially bone white, quickly becoming brown to ash gray or lavender on yeast extract agar. The reverse was orange to maroon initially. In most isolates, the medium (especially yeast extract agar) developed either a deep purple or intense maroon diffusible pigment. As the colonies grew and aged, the central mycelial mat often became lavender-brown with a white (growing) margin. The mature cultures stored in polyethylene bags in a refrigerator (5°) un-

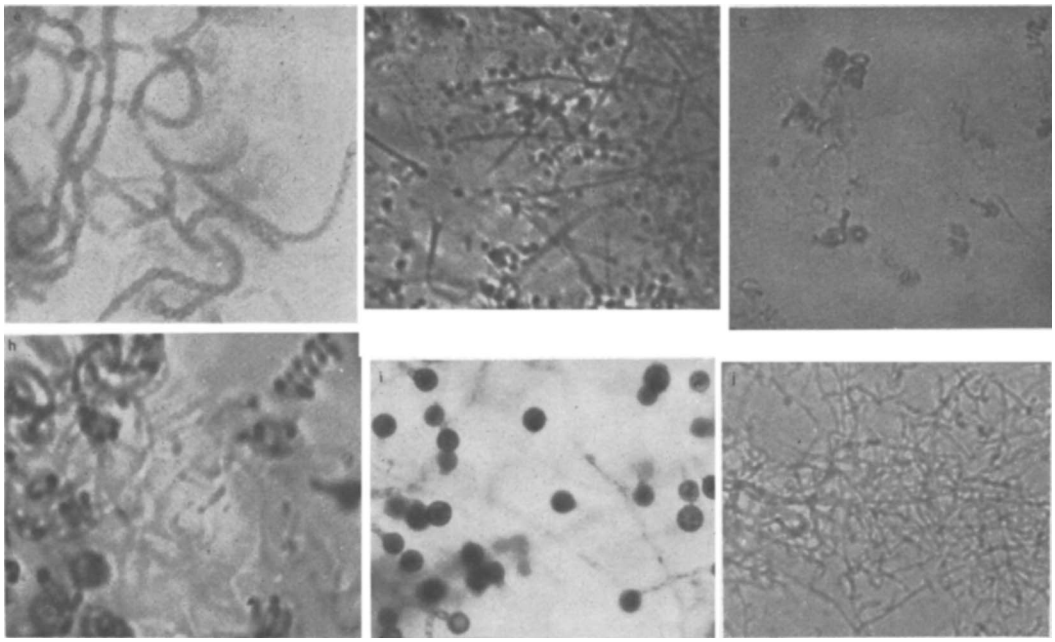

FIG. 1. (e-j) See previous page for caption.

derwent autolysis of the major portion of the surface growth, which became deep purple to almost black in pigmentation. On half-strength nutrient agar, the small central button of aerial mycelium and the vegetative mycelium usually remained unchanged. There was no reaction on peptone iron agar

Streptomyces griseoflavus (Krainsky, 1914) (21); Waksman and Henrici, 1948 was isolated infrequently. The aerial mycelium was velvety and ash gray to white. When ash gray, a white growing margin was present similar to that seen with *S. thermoviolaceus*. The reverse was yellowish brown. Growth was moderate at 4 days when incubated at 40–45° on half-strength nutrient agar. No growth was obtained at 60°.

The aerial hyphae of slide cultures showed monopodial branching with spore chains predominantly in loose spirals (Fig. 1c and h).

Electromicrographs of all isolates showed chains of oval-to-rectangular spores, 0.8–1.0 × 0.4–0.8 μ with nonuniform, spiney projections (Fig. 2c).

Streptomyces fradiae (Waksman and Curtis, 1916) (22); Waksman and Henrici, 1948) was isolated twice, from two separate lots of bagasse. Both isolates were studied in slide cultures on yeast extract agar since aerial mycelium was more abundant on this medium. Growth was good after 4 days of incubation at 40–45°. One isolate did not grow on either yeast extract agar or half-strength nutrient agar at 60° whereas one isolate produced tiny, yellowish colonies on half-strength nutrient agar at 58–60°. The latter had long, open spirals with monopodial branching, but produced no spores. There was no reaction on peptone iron agar.

On yeast extract agar at 45°, the aerial mycelium of one isolate was light pink and the other a grayish pink. The reverse of both isolates was yellow-brown. The aerial mycelium of both isolates showed monopodial branching with loose and regular spirals (Fig. 1d). Spores were ovoid to oblong, 0.8–1.2 × 0.5–0.8 μ and smooth, with slight surface irregularities in some as shown in Fig. 2f.

S. thermovulgaris Henssen, 1957 (20) was isolated from two separate lots of stacked bagasse. Growth was good at 45° on half-strength nutrient agar, producing an abundant aerial mycelium of lavender-brown color with little aerial mycelium at 60°. No soluble pigment was produced. Slide cultures incubated at 45° for 4 days revealed the aerial

production of some loose spirals with monopodial branching but most spore chains were in regular, tight spirals as shown in Fig. 1g. Spores were oblong to ovoid, 1.0–1.2 × 1.4–1.6 μ. Electronmicrographs revealed the spores to be essentially smooth with frequent slight surface irregularities (Fig. 2b).

Streptomyces olivaceus (Waksman 1919) (23); Waksman and Henrici, 1948, was not a common isolate and was rarely found in

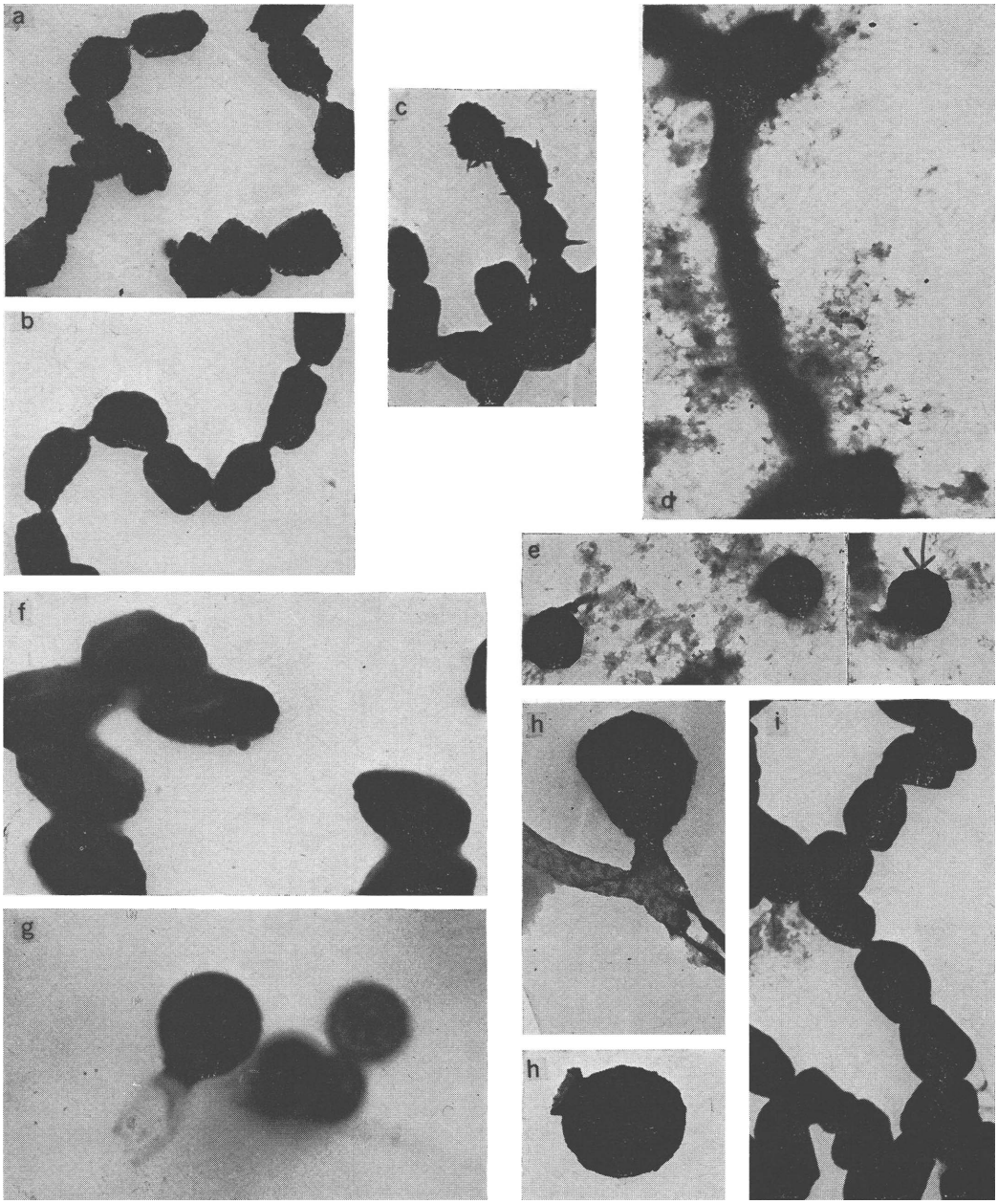

FIG. 2. (a) *S. thermoviolaceus* spores, × 11,000; (b) *S. thermovulgaris* spores, × 14,000; (c) *S griseoflavus* spores, × 11,000; (d) *M. vulgaris*, × 14,000; (e) *M. vulgaris*, spores × 11,000; (f) *S. fradiae* spores, × 17,000; (g) *H. lanuginosa* spores, slide culture; (h) *H. lanuginosa* spores, × 4,750; (i) *S. olivaceus* spores, × 14,000.

old moldy bagasse. It grew rapidly and extensively at 40–45° on yeast extract agar. Aerial mycelium was abundant but short. The surface of the colonies varied from ash gray or ash lavender to bone white. These colors were often mixed. The surface contour also varied from rugose to velvety. There was no growth at 60°. Aerial hyphae were straight or flexuous for the most part with monopodial branching. a few loose spirals were formed. Spores were oval to cylindrical with slightly irregular but smooth surfaces, 0.9–1.0 × 0.4–0.8 μ (see Fig. 2i). The reverse of our isolates varied from yellow to orange. There was no reaction on peptone iron agar.

Humicola lanuginosa (Tsiklinsky 1899; Griffin and Maublanc 1911) (24) Bunce, 1961 was isolated on numerous occasions from both old, dry bagasse and relatively recently stacked material. It was the most common isolate of thermophilic true fungi. It grew abundantly between 45 and 50° and sparsely between 55 and 60° on yeast extract agar.

Colonies were white, cottony, and felt-like during early growth, becoming gray-brown later. Aerial mycelium was abundant. Hyphae were 1.5–4 in diameter. Aleuriophores were at right angles to the hypha and bore colorless spores when young but dark brown with reticulation when mature. A slide culture is shown in Fig. 1i and more detailed spore morphology in Fig. 2g and h.

A common isolate from one lot of bagasse conformed to the general description of *Pseudonocardia* Henssen, 1957 (20). These isolates were lost after storage in a refrigerator at 5° before electron micrographs and physiologic studies could be done. Most of the mycelium was submerged and septate. Aerial mycelium was not recogniezd grossly, but slide cultures revealed aerial branches of the substrate that broke up into chains of oval-to-rectangular spores that appeared to contain more spores in series than the sporulation of substrate hyphae. These are shown in Fig. 1b. We have no basis for separating these isolates from thermotolerant *Nocardia*.

Colonial growth was good on half-strength nutrient agar, giving rise to ivory or lemon-yellow translucent, globose colonies of 1–4 mm in diameter after 24 to 48 hr of incubation. Growth was similar on yeast extract agar at 45–47°, but usually more restricted and without aerial mycelium. Aerial mycelium was not observed on either medium at 58–60°.

Two isolates from relatively fresh bagasse conformed closely to the description of *Thermopolyspora polyspora* Henssen, 1957 (20); Lechevalier *et al.* (26); Cross *et al.* (27) and the progeny of the isolate given the same name (in pure culture) by Corbaz, Gregory, and Lacey (19). There was no difficulty in securing isolation in pure culture (which Henssen was unable to do), but after initial subculturing and harvesting, the isolate was lost under refrigeration. Consequently, we are unable to assign any taxon to our two isolates. It is of interest that chromatographic analysis of whole-cell hydrolysates of one of our isolates and isolate CBS 100:63 (Corbaz *et al.*) revealed a type II (Micromonospora) composition for both. If our chromatographic studies are correct and the classification of Becker, Lechevalier, and Lechevalier (13) is accepted, then neither the isolate of Corbaz et al. (CBS 100:63) nor our isolate subjected to chromatography can be classified as Micropolyspora.

Discussion. Our preliminary survey of thermophilic atinomycetes isolated from samples of bagasse is not conceived as an extensive study in mycology. Not all single isolates were subjected to taxonomic study and particular attention was given to those organisms that had been isolated from moldy hay by others (19). However, fresh bagasse contained few thermophilic eumycetes, actinomycetes, and bacteria, while compressed bales stored under tin roofs in the open field and subjected to high temperatures that at times led to spontaneous combusion were rich in thermophilic microorganisms. This remarkable similarity between exposed bagasse and "moldy" hay flora (19) suggests that they provide similar actinomycete growth environment. Farmer's lung hay antigens, primarily contained in *T. polyspora* (26, 27) develop in wet hay shortly after baling. They are associated with decrease in pH from 4.5 to near 7, content of soluble and volatile

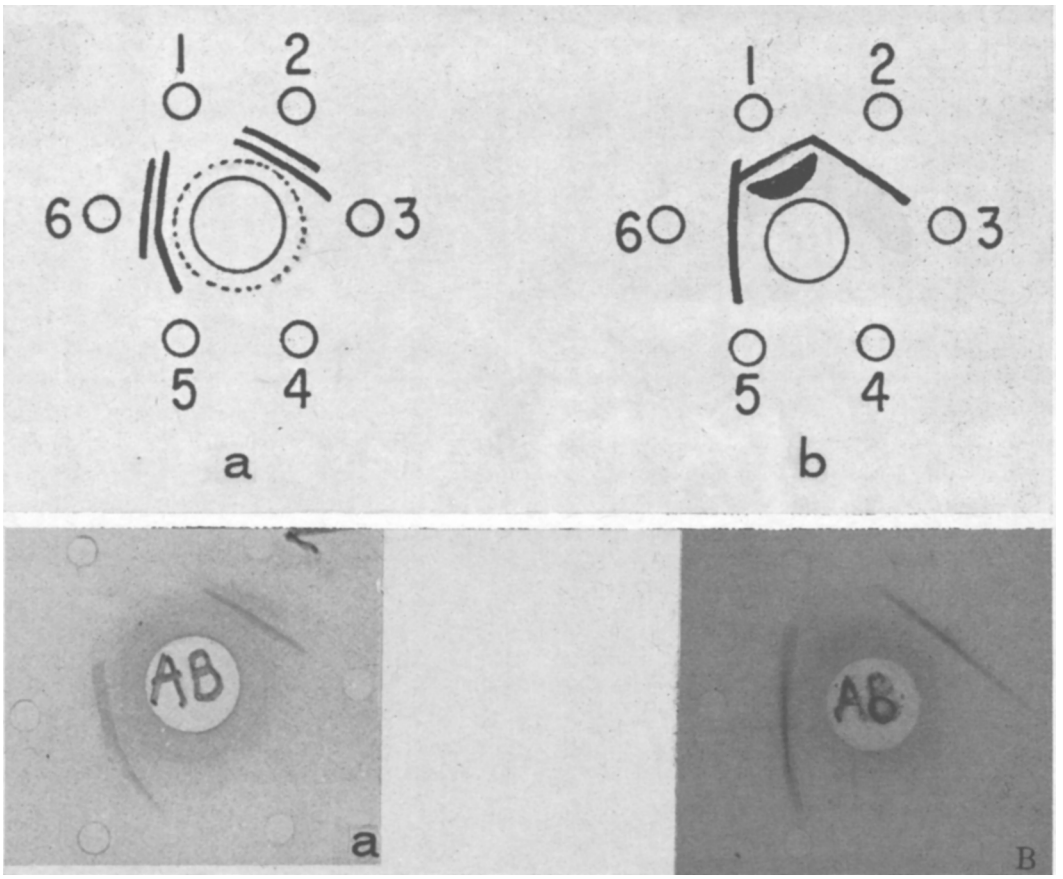

FIG. 3. A and b, Precipitin reactions between sera of two patients with bagassosis and .018 NaCl extracts of several thermophilic actinomycetes. Human antibagasse sera (AB) in center wells. Peripheral wells 2 and 6 contain *M. vulgaris* antigens. Wells 3, 4 and 5 contain *S. olivaceus, S. fradiae,* and *S. thermovulgaris* antigens respectively. Well 1 on right contains a sodium chloride, sodium bicarbonate, phenol alkaline extract (Coca) of crude moldy bagasse and on left *S. griseoflavus* antigen.

nitrogen and numbers of actinomycetes, bacteria, and fungi (28). Although no attempts were made at quantitating colonies, *M. vulgaris, H. lanuginosa,* and *S. thermoviolaceus* were the most common isolates from exposed bagasse samples, including dry specimens from Puerto Rico of pH 5.5–6.0 that had been stored in plastic bags up to 4 years.

More than 50% of the sera of patients with active or recently active bagassosis tested to date demonstrate well-defined precipitin arcs against extracts of the thermophilic actinomycetes *M. vulgaris* but not against the other commonly isolated thermophilic actinomycetes and fungi (Fig. 3a and b).

Individuals who raise pigeons also frequently develop a respiratory disease with symptoms identical to those noted in bagassosis and farmer's lung and their sera demonstrate precipitin arcs against extracts of unknown antigens in pigeon droppings, sera, and feathers on Ouchterlony double-diffusion analysis (3, 8). Fresh scrapings from pigeon coops[1] were cultured in identical manner to our bagasse samples (see *Materials and Methods*), and all plates yielded rich growth in *M. vulgaris*.

Some individuals with pigeon breeder's disease also demonstrate precipitin arcs against *M. vulgaris* extracts (3). However, in our

[1] Supplied by Dr. J. Fink, Milwaukee.

hands most sera of patients with this disease did not react against *M. vulgaris* isolates and it is likely that this organism contains only a few of the many probable antigens of pigeon breeder's disease.

Although *M. vulgaris* is one source of moldy hay antigen in farmer's lung, *T. polyspora* (26, 27) has been reported to be the main source. The taxonomic position of our bagasse isolate resembling *"T. polyspora"* and CBS 100:63 is unsettled since *T. polyspora* may not be a legitimate classification (impure culture) and whole cell hydrolysates are of type II rather than type IV. We do not consider our *"T. polyspora"* isolates, regardless of their taxonomic position as relevant to the etiology of bagassosis for two reasons: (1) they were not isolated from old, moldy bagasse, and (2) they were not antigenically reactive with sera from patients with bagassosis to a significant degree.

In addition, bagassosis results almost exclusively from inhalation of finely dispersed dried sugar cane fiber that has been stored in compressed bales for months or years and *M. vulgaris* has been repeatedly cultured from this type of bagasse.

Immunoelectrophoretic analysis of *M. vulgaris* antigens using rabbit antisera and sera of affected patients should be helpful in detecting specific determinants associated with human disease as well as those shared with related microorganisms and such studies are in progress.

Summary and Conclusion. Industrial workers who grind raw sugar cane fiber from which the sucrose content has been extracted and which has been stored in bales under high environmental temperature develop a characteristic respiratory illness called bagassosis. Precipitins against unknown antigens in crude bagasse have been detected in the sera of many patients with this disorder, and thermophilic microorganisms are suspected as likely sources of bagasse antigen. This report describes the most common thermophilic actinomycetes and fungi isolated from the "bagasse" fiber. These include *M. vulgaris, S. thermoviolaceus, S. griseoflavus, S. fradiae, S. thermovulgaris, S. olivaceus,* and *H. lanuginosa.* The sera of many patients with bagassosis contained precipitins against cellular extracts of *M. vulgaris.* Since bagassosis results almost exclusively from inhalation of dried stored sugar cane fiber and *M. vulgaris* has been the most common isolate from this type of bagasse, it is postulated that *M. vulgaris* is the main source of "moldy bagasse" antigens.

1. Buechner, H., G. P. 35, 108, (1967).
2. Editorial. Brit. Med. J. 2, 713, (1967).
3. Barboriak, J. J., Fink, J. N., Knoblock, H. W., Jr., and Sosman, A. J., Proc. Soc. Exptl. Biol. Med. 125, 991 (1967).
4. Salvaggio, J. E., Buechner, H. A., Seabury, J. H., and Arquembourg, P., Ann. Internal Med. 64, 4 (1966).
5. Kobayashi, M., Stahmann, M., Rankin, J., and Dickie, H., Proc. Soc. Exptl. Biol. Med. 113, 472 (1963).
6. Pepys, J., Riddell, R., Citron, K., and Clayton, M., Thorax 17, 366 (1962).
7. Emanuel, D., Lawton, B., and Wenzel, F., New Engl. J. Med. 266, 333 (1962).
8. Fink, J., Barboriak, J., and Sosman, A., J. Allergy 39, 214 (1967).
9. Pepys, J., Jenkins, P., Lachman, P., and Mahon, W., J. Endocrinol. 3, viii (1965).
10. Bringhurst, L. S., Byrne, R. N., and Gershon-Cohen, J., J. Am. Med. Assoc. 171, 15 (1959).
11. Blackburn, C. R. and Green, W., Lancet 2, 1396 (1966).
12. Cohen, H. I., Merigan, T. Kosek, J., and Eldridge, F., Am. J. Med. 43, 785 (1967).
13. Becker, B., Lechevalier, M., and Lechevalier, H., Appl. Microbiol. 13, 236 (1965).
14. Becker, B., Lechevalier, M., Gordon, R., and Lechevalier, A., Appl. Microbiol. 12, 42 (1964).
15. Hoare, D. and Work, E., Biochem, J. 65, 441 (1967).
16. Ettlinger, L., Corbaz, R., and Hunter, R., Experientia 14, 334 (1958).
17. Cooney, D. G. and Emerson, R., "Thermophilic Fungi." W. H. Freeman Co., San Francisco and London (1964).
18. Ørskov, J., Investigations into the morphology of the ray Fungi. Levin and Munksgaard, Købehavn (1923).
19. Corbaz, R., Gregory, P., and Lacey, M., J. Gen. Microbiol. 32, 449 (1963).
20. Henssen, A., Arch. Mikrobiol. 26, 373 (1957).
21. Krainsky, A., Parastitenk 41, 684 (Abstract II) (1914).
22. Waksman, S. A. and Curtis, R. E., Soil Science 1, 99 (1916).

23. Waksman, S. A., Soil Science 8, 168 (1919).
24. Griffon, E. and Maublanc, A., Bull. Soc. Mycol. France 27, 68 (1911).
25. Tsiklinsky, R., Ann. Inst. Pasteur 13, 500 (1899).
26. Lechevalier, H., Solotorovsky, M., and McDurmont, C., J. Gen. Microbiol. 26, 11 (1961).
27. Cross, T., Maciner, A. W., and Lacey, J., J. Gen. Microbiol. 50, 351 (1968).
28. Gregory, P. H., Festenstein, G. N., Lacey, M. E., and Skinner, F. A., J. Gen. Microbiol. 26, 429 (1964).

Received May 13, 1968. P.S.E.B.M., 1968, Vol. 129.

Heterogeneity of Antisera to Human Growth Hormone, Demonstrated by the Immunofluorescence Technique* (33320)

R. NAYAK,[1] ELEANOR E. MCGARRY, AND J. C. BECK
McGill University Clinic, Royal Victoria Hospital, Montreal, Canada

Earlier studies (1) showed the fluorescein-conjugated antisera to human growth hormone (HGH) localized in acidophils of human, rat, and bovine pituitary glands, and that the fluorescence, which was inhibited when the specific antigen or the unconjugated antisera was applied before staining, was specific.

During inhibition studies, one batch of anti-HGH (titer, 1/12,000; batch HGH-5) inhibited fluorescence caused by another batch of fluorescein-conjugated anti-HGH (titer 1/25,000; Hg-1) in the human but not the rat pituitary gland. Inhibition was repeated with anti-HGH batch HGH-5 and followed by 1:2 and 1:3 dilution of fluorescein-conjugated anti-HGH Hg-1 (which still produced fluorescence); again, fluorescence was not inhibited in the rat pituitary. However, when conjugated and unconjugated anti-HGH Hg-1 from the same batch were used, staining was completely inhibited in both rat and human pituitaries. The present studies were carried out to determine the cause of this discrepancy.

Materials and Methods. The immunofluorescent-staining method used was described previously (2). Preparation of antisera to HGH from 9 rabbits and 1 guinea pig were used for inhibition experiments. Fluorescein-conjugated anti-HGH Hg-1 and Hgh-5, which were used as staining antisera, produced bright fluorescence in rat, human, and bovine pituitaries. The titers were measured by the bis-diazotized benzidine (BDB)–hemagglutination method.

Results. Two antisera did not inhibit staining in any experiment (Table I and II). In human pituitaries, all others produced inhibition of variable degree. In rat, complete inhibition occurred with only two antisera (Table I). In bovine pituitaries, complete inhibition occurred with only one antiserum. Six antisera were conjugated with fluorescein (Table III); since the titer of antisera that failed to stain was unchanged after conjugation, activity was not destroyed during this procedure. Indirect staining, using fluorescein-conjugated sheep anti–rabbit gamma globulin, showed some degree of fluorescence with all eight antisera (Table IV).

These experiments indicate that different rabbits may produce different antisera to a single antigen. Some antisera with high titers failed to inhibit staining, whereas others with lower titers produced fluorescence. When the same antiserum was used for inhibition and staining, inhibition always occurred and was complete; when different antisera were used, inhibition did not always occur. The two antisera that produced no fluorescence were against the same preparations of the antigen and it is possible that HGH batches 163,

* Supported in part by Grant HD 00511-06 from the USPHS.

[1] Medical Research Fellow of the Medical Research Council of Canada. Some of this material was included in Dr. Nayak's thesis for the Ph.D. degree in Experimental Medicine, McGill University, Montreal, 1966.