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THERMOPHILIC ACTINOMYCETES ASSOCIATED WITH FARMER'S LUNG

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Storing hay too wet often results in spontaneous heating to about 65°C and growth of moulds, the inhaled spores of which may cause farmer's lung. Such hays usually have a predominance of thermophilic and thermotolerant organisms, especially actinomycetes. Of 15 species of actinomycetes isolated from hay, three, Micropolyspora faeni, Thermoactinomyces vulgaris and Thermomonospora viridis, have been implicated in farmer's lung (Pepys, Jenkins, Festenstein, Gregory, Lacey & Skinner, 1963; Barrowcliff & Arblaster, 1968), and precipitins to a fourth species, Actinobifida dichotomica, are reported by Professor Molina. Another species from sugar-cane bagasse, Thermoactinomyces sacchari, causes the clinically similar disease, bagassosis (Lacey, 1971 (a)). Other species so far not implicated in disease are often abundant in hay, so that it is important to be able to recognise species causing farmer's lung.

Identification of actinomycete genera

The genera of actinomycetes are separated primarily on microscopic morphological characters. Electron microscopy of spores or biochemical analysis of cell walls are often used to support the classification. The morphological characteristics of different actinomycete genera are shown in Figure 1 and additional features of those found in hay or straw are listed in Table 1. Confusion is most likely between Thermoactinomyces and the white Thermomonospora isolates and between Micropolyspora and some Actinomadura isolates. Thermoactinomyces from hay (T. vulgaris) usually produce colonies with a dense even growth of aerial mycelium with refractile spores spaced along the aerial hyphae, whereas Thermomonospora colonies have a more granular appearance, with the spores in grape-like heads or densely clustered along the hyphae (Cross & Lacey, 1970). Actinomadura produces colourless colonies typically with the mycelium growing in the agar (substrate mycelium) dividing into rods and coccoid elements and frequently with white aerial mycelium bearing short curved chains of spores. The colonies of Micropolyspora (M. faeni) are usually yellowish or orange, and the substrate mycelium does not fragment and produce straight chains of spores on both aerial and substrate mycelia.

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Identification of actinomycete species

Species within some genera such as Nocardia are differentiated by biochemical criteria, and others are identified by morphological criteria. Genera occurring in hay and straw are mostly represented by single species so that generic identification is usually sufficient. Unfortunately Streptomyces is an exception, because over 700 names have been applied to Streptomyces species, although many are probably synonyms. Type cultures of many species have been redescribed recently using standard criteria (Shirling & Gottlieb, 1966, 1968 (a), 1968 (b), 1969). However, it is possible to classify isolates into sections or species using four groups of characters:

1. Aerial mycelium colour. Seven colour series (white, yellow, green, grey, red, violet and blue) are defined by Tresner and Backus (1963).
2. Sporophore morphology. Are the spore chains straight to flexuous (rectiflexibiles), hooked or looped (retinaculiaperti), in spirals (spirales) or in verticils (verticillati or Streptoverticillium)?
3. Spore surface features. Does the electron micrograph show spores smooth, spiny, hairy or warty?
4. Is a black melanoid pigment produced on peptone iron agar or similar media?

Details of these tests are given by Shirling and Gottlieb (1966), and a classification using them by Hütter (1967). Other criteria sometimes used are colour of the substrate mycelium, soluble pigment production ability to use carbohydrates and temperature range.

The Streptomyces species most common in hay can be defined as follows: Streptomyces albus: Aerial mycelium white, sometimes showing pale shades of pink or yellow after incubation at 40°C; spore chains in tight spirals; spores smooth; melanin pigment not produced; growth better at 25°C than 40°C and none at 60°C. Streptomyces griseus: Aerial mycelium buff to pale yellow green (yellow series); spore chains straight to flexuous; spores smooth; melanin pigment not produced; growth better at 25°C than 40°C and none at 60°C.

Species implicated in farmer's lung and similar disease

Micropolyspora faeni (Cross, Maciver & Lacey, 1968), previously erroneously called Thermopolyspora polyspora (Corbaz et al., 1963), is probably the most important cause of farmer's lung disease (Pepys et al., 1963). It grows abundantly in damp self-heating hay stored containing more than 35% water that has heated to 40-65°C (Festenstein, Lacey, Skinner, Jenkins & Pepys, 1965). M. faeni has also been isolated from lung biopsy material (Wenzel, Emanuel, Lawton & Magnin, 1964), sputum (Moore in Lacey & Lacey, 1964),

mushroom compost (Fergus, 1964), mouldy silage, grain, sugar cane bagasse (Cross et al., 1968 (a)), and the air of farm buildings (Lacey & Lacey, 1964).

It can be isolated on half-strength nutrient agar (Corbaz et al., 1963) by incubating inoculated plates at 40°C or 60°C. Colonies are easily suppressed by large numbers of spreading bacteria or Thermoactinomyces vulgaris. The colonies are small, yellowish, sometimes with sparse white aerial mycelium and grow well and characteristically on V-8 juice agar and yeast extract agar.

Substrate hyphae (0.5 - 0.8 µm diameter) are branched and penetrate the agar, forming compact orange-yellow to yellow-brown colonies. Short chains of spores are formed on and below the surface of the agar (Figure 2).

Aerial hyphae (about 1 µm diameter) are usually short and tufted, and the chain of spores formed laterally and terminally give a spiky appearance (Figure 3).

Spore chains are five to ten spores long and their arrangement is shown well in 'Stereoscan' electron micrographs (Figure 4). Transmission electron micrographs show the spores are round to oval, with a smooth or irregular surface, and 0.7 to 1.3 µm long (Figure 5).

No melanin pigment is produced on peptone iron agar, but a brown soluble pigment is sometimes produced. Growth occurs between about 37°C and 60°C and is good between 40°C and 55°C.

Thermoactinomyces (Micromonospora) vulgaris (Tsiklinsky, 1899) is widespread in soil, mouldy fodders, grain, mushroom compost and other self-heating material (Cross et al., 1968 (b)). It grows in hay in similar conditions to Micropolyspora faeni which it sometimes suppresses on isolation plates. On half-strength nutrient agar at 60°C it produces fast growing chalky white colonies with abundant aerial mycelium. Fewer colonies grow at 40°C.

Substrate hyphae are 0.6 to 0.8 µm diameter, branching and penetrating the agar to form a flat spreading colony. Spores are usually sessile and single along the sides of the hyphae (Figure 6). The colony reverse varies from white through to brown.

Aerial hyphae, about 1 µm diameter, frequently branch out from hyphae that grow up from the substrate mycelium and then arch back into the agar. Spores form, as on the substrate hyphae (Figure 7). The aerial mycelium is usually white, but may develop a pink or buff tint.

Spores appear as angular silhouettes in transmission electron micrographs (Figure 8) because of the network of ridges over the surface that are seen better in 'Stereoscan' electron micrographs (Figure 9) or carbon replicas. They have a structure (Figure 10) and heat resistance similar to

bacterial endospores (Cross et al., 1968 (b)) and also resemble these in containing dipicolinic acid. The logarithmic death time at 100°C ($D100^{O}$) is about 1 hour (Cross, Walker & Gould, 1968 (b)). No melanin pigment is produced on peptone iron agar.

Growth occurs between 35°C and 65°C and is optimum at 55°C to 60°C.

Thermomonospora viridis (Schuurmans, Olson & San Clemente, 1956; Küster & Locci, 1964) (=Thermopolyspora glauca, Corbaz et al., 1963) forms characteristic blue-green colonies. It is less thermophilic than Thermoactinomyces vulgaris and M. faeni but grows with them in hay (Festenstein et al., 1965) and also in grain, straw, sugar cane bagasse, peat and soil (Küster & Locci, 1964; Lacey, 1971 (a,b)).

The substrate mycelium consists of branched hyphae 0.6 to 1.0 µm diameter penetrating the agar, but not deeply, and producing few or no spores. The colony reverse is dark blue-green although in some isolates this may be obscured by a brown soluble pigment.

The bluish grey-green aerial hyphae (about 1 µm diameter) bear densely packed oval spores along much of their length (Figure 11). The spores are usually formed singly, but pairs have sometimes been reported (Küster & Locci, 1964) (Figure 12).

The spores are oval, 0.8 - 1.3 x 0.6 - 0.8 µm, with the surface varying from smooth to warty or spiny (Figure 13).

Melanin pigment is not produced on peptone iron agar. Growth occurs between 25°C and 60°C, but most isolates grow best between 40°C and 55°C.

Actinobifida dichotomica (Krassilnikov & Agre, 1964) has been isolated from soil and mushroom compost but not hay.

Substrate and aerial hyphae are 0.7 to 0.8 µm diameter, bearing single spores on dichotomously branches sporophores (Figure 14). Colonies are pale to lemon yellow above, and in reverse, lemon yellow to deep orange.

Spores are angular, resembling Thermoactinomyces vulgaris, and have the structure and properties of bacterial endospores (Cross et al., 1968 (b); Cross & Lacey, 1970).

Thermoactinomyces sacchari (Lacey, 1971) can cause symptoms of bagassosis, a disease clinically resembling farmer's lung. As yet it has been reported only from sugar-cane bagasse. It grows poorly on half strength nutrient agar but well on yeast extract agar at 55°C (Lacey, 1971 (a)).

Substrate hyphae are 0.6 to 0.8 µm diameter, branching and penetrating the agar, on suitable media forming irregularly ridged fast-growing colonies. Spores are formed laterally on short sporophores (Figure 16). Colony reverse colourless at first becoming cartridge buff with sporulation.

Aerial hyphae are about 1 µm diameter, short tufted with spores formed on short sporophores laterally and terminally, but are not always formed (Figure 15). When present aerial mycelium is white, but usually autolyses in a few days to leave the spores in a thick layer on the agar surface.

Spores are angular with a structure like that of a bacterial endospore and similarly heat resistant (Lacey, 1971 (a)).

Melanin pigment is not produced on peptone iron agar but a yellow brown pigment is produced on some media.

Growth occurs from 35°C to 65°C, and is best between 55°C and 60°C.

Summary

Precipitins to four species of actinomycetes (Actinobifida dichotomica, Micropolyspora faeni, Thermoactinomyces vulgaris, Thermomonospora viridis) have been found in patients with farmer's lung disease. Another species (Thermoactinomyces sacchari) may cause the clinically similar disease bagassosis. The characters of these species are described, together with ways in which they can be differentiated from other species that are often numerous in hay or straw.

Résumé

Chez les patients présentant un poumon du fermier des précipitines contre 4 actinomycètes ont été démontrées (Actinobifida dichotomica, Micropolyspora faeni, Thermoactinomyces vulgaris, Thermomonospora viridis). Il est possible qu'une autre espèce (Thermoactinomyces sacchari) soit responsable de la Bagassose. Les caractères de ces actinomycètes et la façon de les distinguer des nombreuses autres espèces trouvées dans le foin et la paille sont discutés.

Zusammenfassung

Bei an Farmerlunge erkrankten Patienten wurden Präzipitine gegen 4 Aktinomycetenarten gefunden (Aktinobifida dichotomica, Mikropolyspora faeni, Thermoaktinomyces vulgaris, Thermomonospora viridis). Eine andere Species (Thermoaktinomyces sacchari) ist möglicherweise für die Bagassosis verantwortlich. Die Eigenschaften dieser Aktinomycete werden beschrieben, und die Möglichkeit ihrer Unterscheidung von den zahlreichen andern in Heu und Stroh vorkommenden Arten diskutiert.

Bibliography

BARROWCLIFF, D.J. & ARBLASTER, P.G.: Farmer's lung: a study of an acute early fatal case. Thorax, 23, 490, 1968.

CORBAZ, R., GREGORY, P.H. & LACEY, M.E.: Thermophilic and mesophilic

actinomycetes in mouldy hay. J.gen.Microbiol. 32, 499, 1963.
CROSS, J. & LACEY, J.: Studies on the genus Thermomonospora. In: Prauser, H. (Ed.): The Actinomycetales. Gustav Fischer, Jena, 211, 1970.
CROSS, T., MACIVER, A.M. & LACEY, J.: The thermophilic actinomycetes in mouldy hay: Micropolyspora faeni sp. nov. J.gen.Microbiol. 50, 351, 1968 (a).
CROSS, T., WALKER, P.D. & GOULD, G.W.: Thermophilic actinomycetes producing resistant endospores. Nature, Lond. 220, 352, 1968 (b).
FERGUS, C.L.: Thermophilic and thermotolerant molds and actinomycetes of mushroom composts during peak heating. Mycologia, 56, 267, 1964.
FESTENSTEIN, G.N., LACEY, J., SKINNER, F.A., JENKINS, P.A. & PEPYS, J.: Self-heating of hay and grain in dewar flasks and the development of farmer's lung antigens. J.gen.Microbiol. 41, 389, 1965.
HÜTTER, R.: Systematik der Streptomyceten unter besonderer Berücksichtigung der von ihnen gebildeten Antibiotica. Bibl.Microbiol. S. Karger, Basle / New York, 6, 1967.
KRASILNIKOV, N.A. & AGRE, N.S.: A new actinomycete genus - Actinobifida n. gen. yellow group - Actinobifida dichotomica n. sp. Mikrobiologiya, 33, 935, 1964.
KÜSTER, E. & LOCCI, R.: Transfer of Thermoactinomyces viridis (Schuurmans et al., 1956) to the genus Thermomonospora as Thermomonospora viridis (Schuurmans, Olson & San Clemente) comb. nov. Int.Bull.bact. Nomencl. 13, 213, 1963.
LACEY, J.: Thermoactinomyces sacchari sp. nov., a thermophilic actinomycete causing bagassosis. J.gen.Microbiol. 66, 327, 1971 (a).
LACEY, J.: The microbiology of moist barley storage in unsealed silos. Ann.appl.Biol. 69, 187, 1971 (b).
LACEY, J. & LACEY, M.E.: Spore concentration in the air of farm buildings. Trans.Br.mycol.Soc. 47, 547, 1964.
LECHEVALIER, H.: The actinomycetes. In: Heukelekian, H. & Dondero, N.C. (Eds.): Principles and applications in aquatic microbiology. John Wiley, New York, 1964.
PEPYS, J., JENKINS, P.A., FESTENSTEIN, G.N., GREGORY, P.H., LACEY, M.E. & SKINNER, F.A.: Farmer's lung: thermophilic actinomycetes as a source of "farmer's lung hay" antigen. Lancet, I, 607, 1963.
SCHUURMANS, D.M., OLSON, B.H. & SAN CLEMENTE, C.L.: Production and isolation of Thermoviridin, antibiotic produced by Thermoactinomyces viridis n. sp. Appl.Microbiol. 4, 61, 1956.
SHIRLING, E.B. & GOTTLIEB, D.: Methods for characterization of Streptomyces species. Int.J.Syst.Bact. 16, 313, 1966.
SHIRLING, E.B. & GOTTLIEB, D.: Co-operative description of type cultures of Streptomyces II. Species descriptions from first study. Int.J. Syst.Bact. 18, 69, 1968 (a).
SHIRLING, E.B. & GOTTLIEB, D.: Co-operative description of type cultures of Streptomyces III.Additional species descriptions from first and second studies. Int.J.Syst.Bact. 18, 279, 1968 (b).
SHIRLING, E.B. & GOTTLIEB, D.: Co-operative description of type cultures of Streptomyces IV. Species descriptions from the second, third and fourth studies. Int.J.Syst.Bact. 19, 391, 1969.

TRESNER, H.D. & BACKUS, E.J.: System of Colour Wheels for Streptomycete Taxonony. Appl.Microbiol. 11, 335, 1963.
TSIKLINSKY, P.: Sur les Mucedinées thermophiles. Ann.Inst.Pasteur, 13, 500, 1899.
WENZEL, F.J., EMANUEL, D.A., LAWTON, B.R. & MAGNIN, G.E.: Isolation of the causative agent of Farmer's Lung. Ann.Allergy, 22, 533, 1964.
WILLLIAMS, S.T., DAVIES, F.L. & CROSS, T.: Identification of genera of the Actinomycetales. In: Identification methods for microbiologists, part B. Soc.appl.Bact.Tech.Series, 2, 111, 1968.

TABLE 1 CHARACTERISTICS OF ACTINOMYCETE GENERA FROM HAY AND STRAW

Genus	Substrate mycelium = SM	Aerial mycelium = AM	Spore formation	Spore morphology	Cell wall type*
Thermoactinomyces	Not fragmenting, white-brown	White-buff or pinkish	Spores sessile or sporophores short simple. Spores solitary on AM and SM	Angular	3
Actinobifida	Not fragmenting, yellow-orange or brown or white	Yellow, brown or white	Sporophores dichotomously branched. Spores solitary on AM and SM or AM only	Angular, round or oval	3
Thermomonospora	Not fragmenting, white-yellow or dark blue green	White or blue	Sporophores simple or dichotomously branched. Spores single on AM and SM or AM only.	Oval, smooth-warty - spiny	3 or 4
Actinomadura	Fragmenting, white	White	Spores in short chains often curved on AM only	Round ± smooth	3
Nocardia	Fragmenting, white, yellow, orange, red	White or absent	Spores generally not formed	-	4
Micropolyspora	Sometimes slight fragmentation, yellowish	White	Spores in short lateral and terminal chains on AM and SM	Round - oval, ± smooth	4
Streptomyces	Not fragmenting, various colours	White, yellow, grey green, red, violet, blue	Spores in long chains, straight, flexuous or in spirals on AM only	Oval, smooth, spiny, hairy or warty	

*) after Lechevalier & Lechevalier (1970)

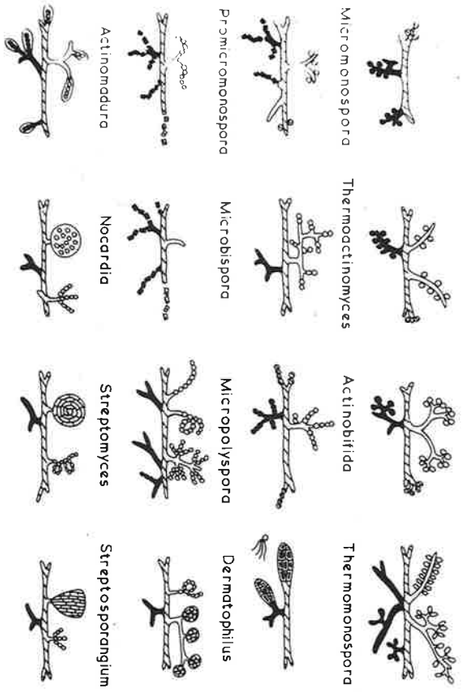

Figure 1: Morphology of actinomycete genera. Hyphae growing in air, unshaded; on surface of agar, shaded; within agar, black (After Lechevalier, 1964; Williams, Davies & Cross, 1968).

Figures 2 - 5: Micropolyspora faeni
Figure 2: Substrate hyphae and spores (1 : 650)
Figure 3: Aerial hyphae and spores (1 : 550)
Figure 4: Scanning electron micrograph of spores (1 : 2100)
Figure 5: Transmission electron micrograph of spores (1 : 28,500)

Figures 6 -10: Thermoactinomyces vulgaris
Figure 6: Spores on substrate hyphae (1 : 650)
Figure 7: Aerial hyphae and spores (1 : 1300)
Figure 8: Transmission electron micrograph of spores (1 : 28,500)
Figure 9: Scanning electron micrograph of spores (1 : 33,600)
Figure 10: Sectioned spore (1 : 66,000)

Figures 11-13: Thermomonospora viridis
Figure 11: Spores on aerial hyphae (1 : 780)
Figure 12: Scanning electron micrograph of spores on aerial hyphae (1 : 6,300)
Figure 13: Transmission electron micrograph of spores (1 : 28,500)

Figure 14: Actinobifida dichotomica. Aerial hyphae and spores (1 : 1,300)

Figures 15-16: Thermoactinomyces sacchari
Figure 15: Spores on aerial hyphae (1 : 650)
Figure 16: Spores on substrate hyphae (1 : 1,600)


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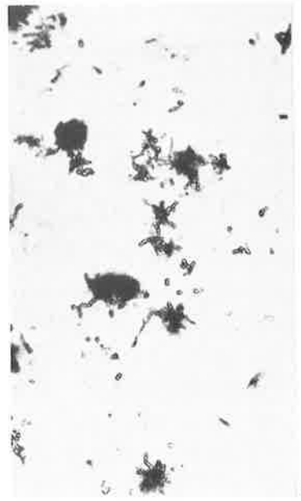
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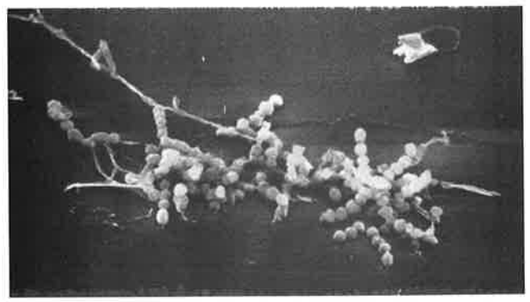
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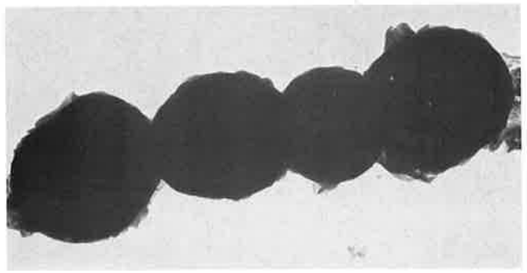
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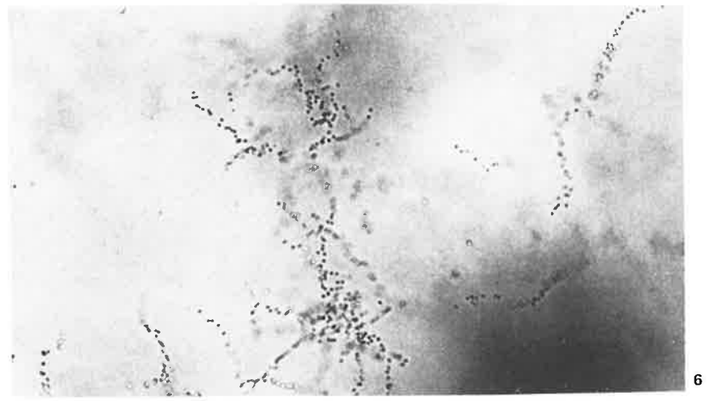

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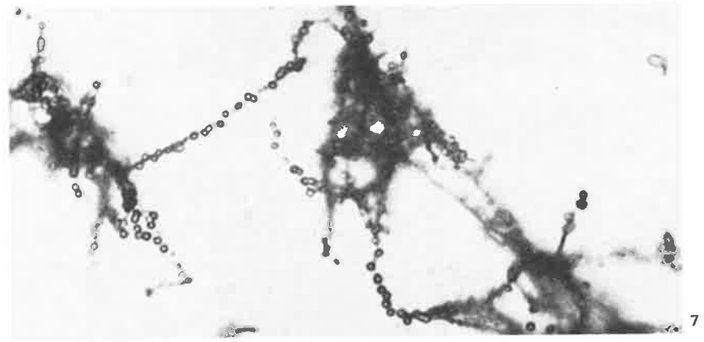

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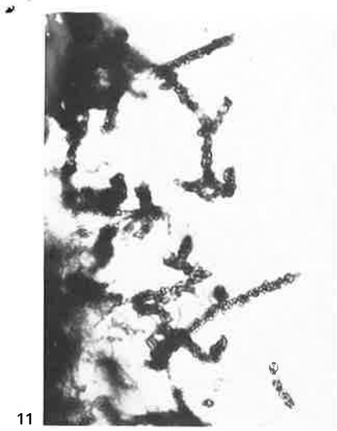

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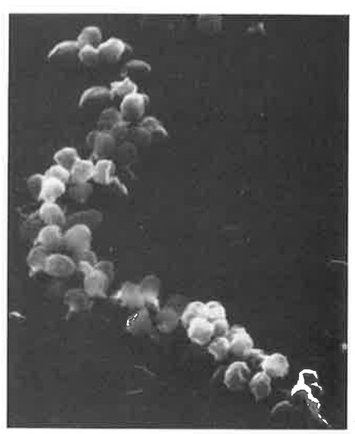

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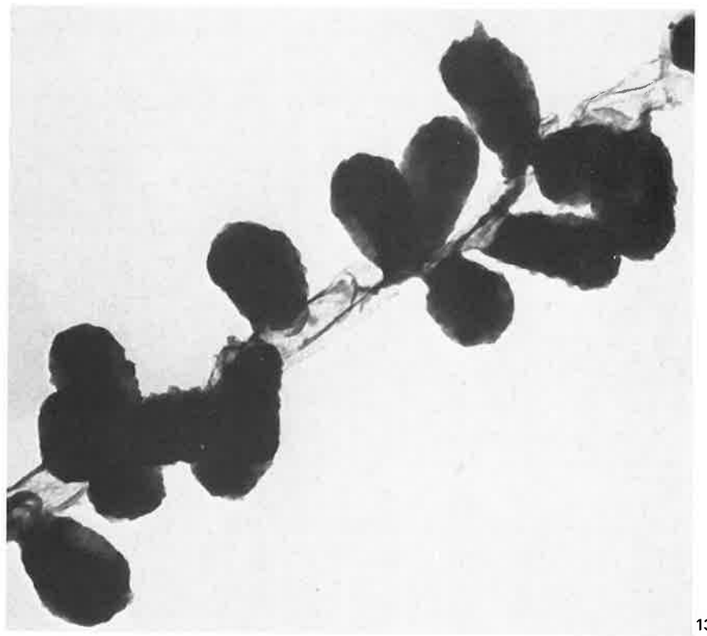

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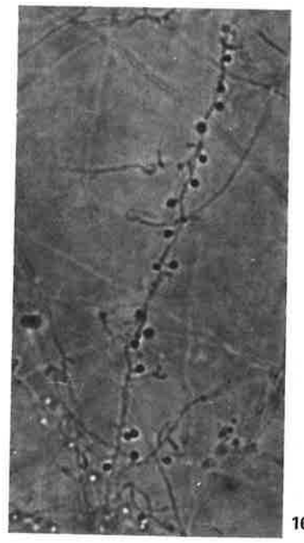

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