

**The Thermophilic Actinomycetes in Mouldy Hay:  
*Micropolyspora faeni* sp.nov.**

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SUMMARY

Taxonomic studies on the thermophilic oligosporic actinomycete chiefly responsible for the respiratory disease Farmer's Lung support the view that the organism was incorrectly identified as *Thermopolyspora polyspora* Hens. The organism belongs to the genus *Micropolyspora* Lechevalier *et al.* and is described as a new species *Micropolyspora faeni*.

INTRODUCTION

Mouldy hay associated with farmer's lung disease contains large numbers of actinomycete spores (Gregory & Lacey, 1962, 1963*a*). The commonest thermophilic and mesophilic species were described by Corbaz, Gregory & Lacey (1963) and spores of two of the thermophilic species named *Thermopolyspora polyspora* Hens and *Micromonospora vulgaris* Waksman *et al.* have been shown to be the main source of 'farmer's lung hay' antigens (Pepys *et al.* 1963; Pepys & Jenkins, 1965).

*Micromonospora vulgaris* Waksman, Umbreit & Cordon (1939) and *Thermoactinomyces vulgaris* Tsiklinsky (1899) are synonymous (Kuster & Locci, 1964) and the latter name is now generally preferred (Festenstein *et al.* 1965). Examination of the thermophilic hay strains named *Thermopolyspora glauca* Corbaz, Gregory & Lacey (1963) show that spores are produced singly on short sporophores borne laterally on the aerial hyphae in a raceme or pallasade-like structure. True spore formation on the substrate mycelium was not observed. These strains must therefore be placed in the species *Thermomonospora viridis* (Schuurmans, Olson & San Clements, 1956) Kuster & Locci (1963).

Since the work of Corbaz, *et al.* (1963) several papers have been published commenting on the taxonomy and descriptions of oligosporic actinomycetes and questioning the validity of the binomial *Thermopolyspora polyspora* Hens (Kosmachev, 1964; Kalakutskii, 1964; Krassilnikov, 1964; Becker, Lechevalier & Lechevalier, 1965; Lechevalier & Lechevalier, 1965; Lechevalier, Lechevalier & Becker, 1966), and Lacey suggested that the organisms isolated from hay should be referred to the genus *Micropolyspora* Lechevalier, Solotorovsky & McDurmont, 1961 (Festenstein *et al.* 1965).

Because of the importance of the organism named *Thermopolyspora polyspora* Hens

in farmer's lung disease and the confusion over the taxonomy of oligosporic actinomycetes, we have made further taxonomic studies on strains isolated from hay and other habitats.

## METHODS

### *Isolation*

Several oligosporic thermophilic actinomycetes were isolated from mouldy hay samples kindly supplied by Dr R. E. Taylor of the National Agricultural Advisory Service, Leeds. Isolations were made by using an Andersen sampler (Andersen, 1958) loaded with plates of either yeast extract isolation agar or half-strength nutrient agar containing 0.5 mg. cycloheximide/ml. to suppress fungal growth. The sampler was connected by a short length of rubber tube to a sterile sample tin (12 × 6 × 4 in.) containing a weighed hay sample. The hay in the tin was shaken (for about 3 min.) to suspend actinomycete spores and the air spora sampled for brief periods (30 sec.) by connecting the Andersen sampler to a small vacuum pump after allowing the larger particles to settle.

From plates incubated at 55°, colonies similar to those identified as *Thermopolyspora polyspora* by Corbaz, Gregory & Lacey (1963) were subcultured and purified on glucose nutrient agar. The organism was isolated frequently from the hay samples studied; 50 isolates were examined in detail. They fell into a well-defined group when using morphology and pigmentation as main criteria. Isolate CUB 58 was chosen for detailed study; its growth rate and pigmentation differed very little from other isolates when grown on a range of media.

Other strains, from hay and grain samples, were isolated with the aid of an Andersen sampler suspended horizontally in a small wind tunnel (Gregory & Lacey 1963*a, b*).

Isolated strains and strains originating from other laboratories (Table 1) were compared with each other and with published descriptions of *Micropolyspora* and *Thermopolyspora* species.

Table 1. *Strains included in the taxonomic study*

Laboratory reference no.	Given name and source of isolate
A 94	<i>Thermopolyspora polyspora</i> . Corbaz, Gregory & Lacey type strain
A 415	<i>T. polyspora</i> isolated from sputum. B. Moore. Exeter
A 444	<i>Micropolyspora brevicatena</i> 1086W. H. A. Lechevalier
A 445	<i>Micropolyspora</i> sp. LL 660. H. A. Lechevalier
A 446	<i>T. polyspora</i> isolated from mushroom compost (Fergus, 1964)
A 447	<i>T. polyspora</i> isolated from lung biopsy material (Wenzel <i>et al.</i> 1964)
A 448	<i>Micropolyspora</i> sp. isolated from moist, overheated barley grain
CUB 58	<i>Micropolyspora</i> sp. isolated from hay
A 443	<i>Nocardia madurae</i> Sal. 1. M. P. Lechevalier (Gordon, 1966)

### *Media*

The two media used for isolations were the yeast extract and half strength nutrient agars used by Corbaz *et al.* (1963). The other media used for characterization were as follows.

*Nutrient agar with glucose.* Nutrient agar (Oxoid) with 1% (w/v) glucose.

*Glucose yeast extract agar.* Glucose 10 g. yeast extract (Difco) 10 g.; K<sub>2</sub>HPO<sub>4</sub>, 0.5 g.; agar 20 g.; distilled water 1 l.; pH 6.8–7.0.

*Casamino acid agar.* Glucose, 3.0 g.; NaNO<sub>3</sub>, 2.0 g.; K<sub>2</sub>HPO<sub>4</sub>, 1.0 g.; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 g.; KCl, 0.5 g.; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.01 g.; Casamino acids (Difco), 1.0 g.; agar, 15 g.; distilled water 1 l.

*Milk agar.* Skim milk powder (Oxoid), 10 g.; agar, 15 g. distilled water, 200 ml. This agar after autoclaving was poured as a thin layer on the surface of solid 2.0% water agar.

*Defined medium.* (Lechevalier, Solotorovsky & McDurmont, 1961).

*V-8 agar* (*SAB Manual*, 1957).

*Oatmeal agar.* ISP (Shirling & Gottlieb, 1966).

*Inorganic salts starch agar.* ISP (Shirling & Gottlieb, 1966).

*Peptone yeast extract iron agar.* ISP (Shirling & Gottlieb, 1966).

*Glycerol asparagine agar.* ISP (Shirling & Gottlieb, 1966).

*Cellulose agar.* 1% (w/v) ball-milled Whatman powdered cellulose in carbon utilization medium ISP (Shirling & Gottlieb, 1966).

*Potato carrot agar.* (Cross, Lechevalier & Lechevalier, 1963).

*Corn meal salts agar* (Cross *et al.* 1963).

*Potato glucose agar* (Baldacci, Comasch, Scotti & Spalla, 1953).

*Chitin agar* (Lingappa & Lockwood, 1962).

*Nutrient agar* (Oxoid).

*Lab Lemco agar* (Oxoid).

#### *Colonial morphology*

Cultures were examined directly on the surface of agar plates by using a ×40 long-working-distance objective or phase microscopy. Slide cultures, prepared by the method of Colmer & McCoy (1950), were dried and stained with Hucker's modified Gram stain. We classified the mycelium into: (1) substrate (primary) mycelium which grows into and forms a compact layer on the surface of the agar medium; (2) aerial (secondary) mycelium which arises from the substrate mycelium and grows into the air away from the agar surface.

Colour determinations, where given, refer to the following codes: P = Prauser selection Baumann Farbtonkarte (Prauser, 1964) CHM = Color Harmony Manual tabs ISCC-NBS 1955 (Tresner & Backus, 1963).

#### *Ability to grow at various temperatures*

Plates to be incubated at high temperatures were placed in incubators containing a tray of water to prevent excessive drying of the agar. For determining the growth temperature range a polythermostat similar in design to that described by Oppenheimer & Drost-Hansen (1960) was used.

### RESULTS

The thermophilic *Micropolyspora* sp. (A445, A448, CUB58) and strains named *Thermopolyspora polyspora* (A94, A415, A446, A447) were very similar in morphology and pigmentation, and grew well on most of the media at 55° and 49°. Colonies are raised and pale orange-yellow to yellow-brown in colour. The substrate mycelium in the agar and forming the colony bears short, straight chains of spores. The white aerial mycelium is short, and bears straight, short chains of spores both laterally and terminally. The strains can be classified within the genus *Micropolyspora* (Lechevalier

*et al.* 1961), are readily differentiated from *Micropolyspora brevicatena* (Table 2), and constitute a new species within the genus.

*Description of Micropolyspora faeni* Cross, Maciver & Lacey *sp.nov.*  
(*faeni*—of *hay*) NCIB9984

*Substrate mycelium.* About 0.5–0.8  $\mu$  in diameter. Hyphae branching, penetrating the agar medium and forming compact colonies which are at first colourless, becoming orange-yellow to yellow-brown. Short chains of spores can be seen on the substrate mycelium forming the colony and also beneath the surface of the agar (Pl. 1, figs. 3–5). Occasionally intercalary spore or chlamydospore formation was observed in slide cultures (Pl. 2, figs. 7, 8).

Table 2. *Differential characteristics of Micropolyspora brevicatena and M. faeni*

Character	<i>M. brevicatena</i> 1086w	<i>M. faeni</i> A94
Growth at 50° and 55°	—	+
Substrate mycelium	Pale pink orange to pink yellow brown (P.Oc2a–Oc4a)	Orange yellow to yellow brown (P.Coo2a–Coo4a)
Spore chains	Flexibilis (flexuous, wavy to slightly coiled)	Rectus (straight, stiff)

*Aerial mycelium.* About 1.0  $\mu$  in diameter, white and abundant on certain media. Short chains of spores are borne on the aerial hyphae both laterally and terminally. The lateral chains are short (about 5 spores) while those at the tip may be longer (about 5–10 spores) (Pl. 1, figs. 1, 2). Spore chains on the substrate and aerial hyphae are very similar morphologically and show a beaded appearance in stained preparations, with conspicuous non-staining areas between each spore (Pl. 2, figs. 9, 10). Spores are globose to oval, sometimes irregular, smooth, 0.7–1.3  $\mu$  long, in electron micrographs (Pl. 2, figs 11, 12).

*Appearance on various media*

Descriptions refer to strain A94 after growth at 55°. Some differences between strains were noted and detailed below.

*Yeast extract agar.* Growth good, substrate mycelium light orange brown (P. Coo2a–Coo4a). Aerial mycelium with spores, developing slowly at the edge of the colony but growing upright and away from the centre of the colony. No soluble pigment.

*V-8 agar.* Growth good, substrate mycelium light orange-brown (P. Coo2a–Coo4a). Aerial mycelium production good, pale orange-yellow to pearl pink shell (CHM 3ca) initially becoming white on further incubation. Chains of spores (4–5 spores) on substrate mycelium after 2 days. Aerial mycelium short and coarse bearing short chains of spores (2–5 spores). Some brown soluble pigment.

*Nutrient agar.* Growth only moderate after 2 days but good after 5 days. Substrate mycelium pale buff (PCo4a) to cream (PCo6a) bearing single spores and chains of spores. Aerial mycelium developing after 3 days, off white to oyster white (CHM b) bearing chains of 5–7 spores. No soluble pigment. The strains exhibited similar characteristics on half strength nutrient agar. On nutrient agar + glucose growth was more rapid and abundant aerial mycelium evident after 2 days incubation.

*Oatmeal agar.* Growth poor, restricted, colourless substrate mycelium bearing spore chains (3–5 spores).

*Lab Lemco agar.* Good growth after 2 days incubation. Substrate mycelium cream buff (P. Coo5a) becoming pale orange brown (PCoo3a) after 5 days. Chains of spores on substrate mycelium. No aerial mycelium.

*Glycerol asparagine agar.* Growth good where inoculum is heavy but elsewhere only isolated colonies grow slowly. Substrate mycelium cream buff (P. Coo5a) becoming lighter on further incubation (P. Co6a) bearing single chains and short chains. Aerial mycelium absent.

*Glucose yeast agar.* Growth abundant after 2 days incubation. Substrate mycelium pale orange brown (P. Coo3m) bearing chains of spores. No aerial mycelium. No soluble pigment.

*Casamino acid agar.* Growth slow at first but appreciable colonies formed after 5 days incubation, light orange brown in colour (P. Coo2a). Short chains of spores on substrate mycelium. No aerial mycelium, except in strains A415, A448, CUB58 where it was sparse, white. No soluble pigment.

*Potato glucose agar.* Growth limited after 2 days becoming moderate after 5 days incubation. Substrate mycelium pale brown (P. Coo4a) becoming paler on further incubation (P. Co6a) and bearing chains of spores. Aerial mycelium developing in patches, off white and bearing chains of spores (2–5–8 spores). No soluble pigment.

*Inorganic salts-starch agar.* Very thin colourless growth. No aerial mycelium. No starch hydrolysis detectable.

*Peptone yeast extract iron agar.* Growth good, pale orange brown with no aerial mycelium. No melanin pigment.

*Potato carrot agar.* Growth poor, pale brown colonies. No aerial mycelium.

*Corn meal salts agar.* Growth thin, pale brown colonies bearing chains of spores. No aerial mycelium.

*Milk agar.* Excellent growth, orange yellow colonies lacking aerial mycelium. No casein digestion.

*Defined medium.* Growth moderate after 3 days. Strains A445, A446, A448 orange yellow; A94, A415 and CUB58 colourless to pale yellow. No aerial mycelium, no soluble pigment.

*Chitin agar.* Strains A94 and A415 no growth. Very thin transparent growth exhibited by strains A445, A446, A447, A448 and CUB58. No clearing of colloidal chitin.

*Cellulose agar.* No growth.

#### *Effect of pH value on growth*

Good growth was obtained on plates of nutrient agar buffered with phosphate over the range pH 6.0, 6.3, 7.4 and 8.1 at 55° and 49°. At 37° good growth was obtained at pH 6.0, moderate growth at pH 6.3 and 7.0, and no growth at pH 8.1. Similar results were obtained for A415. The other strains were less sensitive to pH 8.1 at 37° but growth was always less than at pH 6.0.

#### *Effect of temperature on growth*

*Micropolyspora faeni* is a facultative thermophile or eurithermal thermophile (Gaughran, 1947) exhibiting growth on plates at 55° and on some media at 37°. When incubated on agar slopes placed in a polythermostat, a wide range of growth tempera-

tures were observed (Table 3), the range and extent of growth depending on the nature of the medium. The maximum growth temperatures observed in the polythermostat tubes were lower than those in agar-plate cultures and must reflect differences in aeration and humidity.

#### Occurrence

*Micropolyspora faeni* is a characteristic component of the microflora of very mouldy hays, which may cause farmer's lung disease in susceptible subjects. These are hays that have been baled when too wet and have subsequently heated spontaneously. Water contents in excess of 35% may permit heating to 50–70° with the growth of abundant thermophilic and thermotolerant moulds and actinomycetes (Gregory, Lacey, Festenstein, Skinner, 1963; Festenstein *et al.* 1965). Most *M. faeni* isolates were obtained from hay stored in a Dewar flask at 47% water content which heated to 61°. Hay associated with cases of farmer's lung disease and respiratory disease in cattle yielded up to 133 colonies/g. dry wt hay under standard conditions in the wind tunnel (Gregory & Lacey, 1963*a*). This is equivalent to about 1% of the blowable fraction of the spore load, or about 0.15% of the total (Gregory & Lacey, 1963*b*).

*Micropolyspora faeni* has also been isolated from lung biopsy material (Wenzel, Emanuel, Lawton & Magnin 1964), sputum (Moore, in Lacey & Lacey, 1964), mushroom compost (Fergus, 1964) and from mouldy silage, barley grain, straw and sugar cane bagasse after self-heating, and the air of farm buildings where fodder was being stored or moved.

Table 3. Growth of *Micropolyspora faeni* A94 on slopes in a polythermostat after incubation for 67 hr

Temperature	Half-strength nutrient	Nutrient+ glucose	Glucose+yeast extract	Yeast extract
61.4	—	—	—	—
59.0	—	(+)	—	—
56.7	(+)	(+)	—	—
54.6	+	++	(+)	—
52.3	++	++	+	—
50.4	++	++	++	(+)
48.1	++	++	++	+
46.0	++	++	++	+
44.0	++	++	+	+
42.0	++	++	+	+
40.0	++	++	(+)	(+)
38.2	++	++	(+)	(+)
35.8	+	+	(+)	(+)
34.2	(+)	(+)	(+)	(+)
32.5	(+)	(+)	(+)	—

Key: — no growth, (+) poor growth; + moderate growth; ++ good growth.

#### Pathogenicity

Although *Micropolyspora faeni* has been isolated from sputum and lung biopsy material of farmer's lung patients, there is so far no evidence that it grows in the lung. However, *M. faeni* is a rich source of farmer's lung hay antigen, and corresponding precipitins are found in the sera of affected subjects. Inhalation of extracts of *M. faeni*, or of fractions of the antigen it produces, by affected subjects provokes symptoms of farmer's lung disease (Pepys *et al.*, 1963; Pepys & Jenkins, 1965). Farmer's lung is a

disease of the peripheral parts of the broncho-pulmonary system. The spores of *M. faeni*, being about  $1\ \mu$  diameter, are well suited for deposition in the alveolar region, where the reaction occurs. Since *M. faeni* has been identified as a cause of farmer's lung, this disease has been prescribed under the National Insurance (Industrial Injuries) Act 1946, in Britain.

## DISCUSSION

The genus *Thermopolyspora* was proposed by Henssen (1957) to include organisms forming unbranched aerial hyphae bearing short chains of conidia; her published descriptions and photographs do not have any reference to spores on the substrate mycelium. Her organism *T. polyspora* could therefore be considered as a thermophilic Streptomyces or Nocardia and it is unfortunate that it is now impossible to obtain any of her strains for comparative study. Henssen's descriptions were based on the type of sporulation produced only when the organism was grown in the presence of contaminating bacteria. Because of this, her taxon is considered to be illegitimate under the *International Code of Nomenclature of Bacteria and Viruses* (1958), Rule 24 g. (Becker, *et al.* 1965). However, under the *International Code of Nomenclature of Bacteria* (1966) Henssen's binomial would be valid since her description was based only on the actinomycete component of bacterially contaminated cultures. Also, no bacteria can be seen in her photographs of *T. polyspora*. Isolate A94 has been examined by Dr Henssen, who considers it to be distinct from *T. polyspora* (Henssen 1957). Two further species have been included in the genus *Thermopolyspora* (Krassilnikov & Agre, 1964) and both were reported to produce chains of spores on the aerial hyphae and from the mycelium on the surface of the colony. *Thermopolyspora flexuosa* was reported to produce short spiral spore-chains, and the individual spores to have a spiny membrane. The colonies were pale yellow brown to dark brown and the weakly developing aerial mycelium, white with a blue shade. *Thermopolyspora rectivirgula* had straight chains of smooth spores, colourless to slightly yellow colonies and abundant yellowish aerial mycelium. However, later that same year Krassilnikov (1964) noted that *Thermopolyspora* was a questionable genus. He stated that 'according to fruiting structures, the cultures of this genus do not differ from that of *Micropolyspora*. It is only more thermophilic, which in our opinion is not sufficient for their separation into a separate genus'. *Thermopolyspora flexuosa* K1132 was examined by Becker *et al.* (1965) and found to produce a sporulation similar to that described by Henssen for *T. polyspora*. However, this type of sporulation is also characteristic of sporulating strains of *Nocardia madurae* (Gordon, 1966) (see Pl. 1, fig. 6) and the cell-wall composition of strain K-1132 was that of 'Type 3' actinomycetes which includes *N. madurae*. There is thus some doubt as to whether these two species belong to the genus *Micropolyspora*.

A new species named *Micropolyspora thermovirida* was described by Kosmachev (1964). The organism is a facultative thermophil, bearing spore chains of 1 to 15, occasionally 20, spores and the aerial mycelium is greenish grey. A second new species named *M. caesia* was described by Kalakutskii (1964). Spores produced on the substrate and aerial hyphae are mostly single but occasionally in short chains of two, three or rarely four. The organism is mesophilic and produces a greyish blue aerial mycelium. These species broaden the definition of the genus *Micropolyspora* and their exact taxonomic position requires further investigation. They differ significantly from the properties described for *M. faeni*.

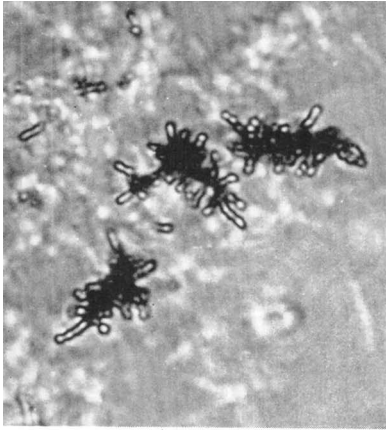
*Micropolyspora faeni* A94 has a cell-wall composition of the Type IV or 'Nocardia type' actinomycetes, which include *Micropolyspora brevicatena* and other *Micropolyspora* strains (Becker, *et al.* 1965). Its morphology and method of spore formation is typical of the genus *Micropolyspora* and it differs from previously described species. It may be significant that *M. brevicatena* was also isolated from sputum. Details of the clinical history of the two patients concerned have not been published, except that they were being treated for tuberculosis. Symptoms of farmer's lung disease have sometimes been mistaken for tuberculosis, and this might suggest that *M. brevicatena* could cause a similar disease.

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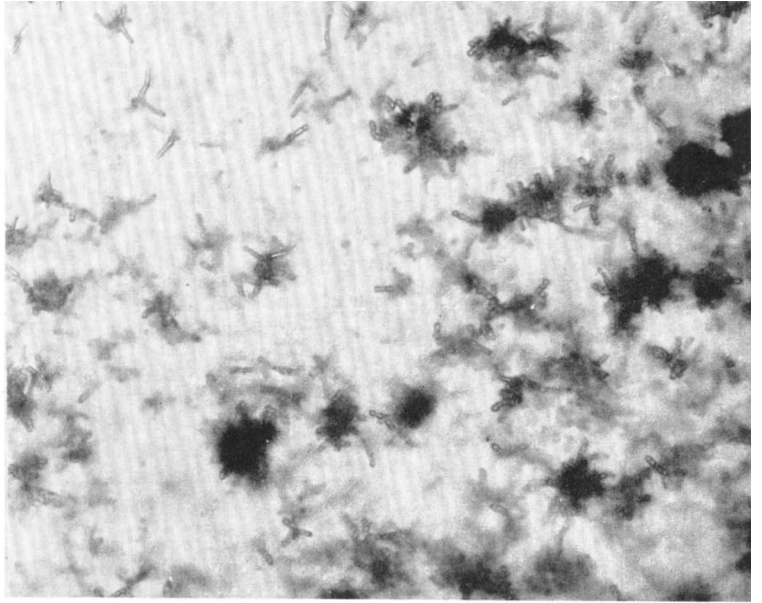
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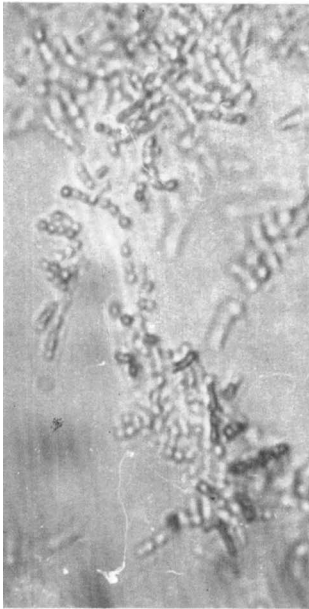




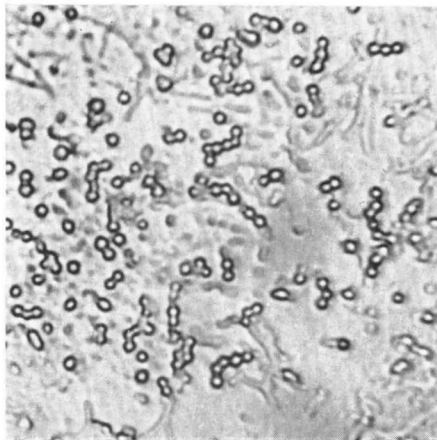
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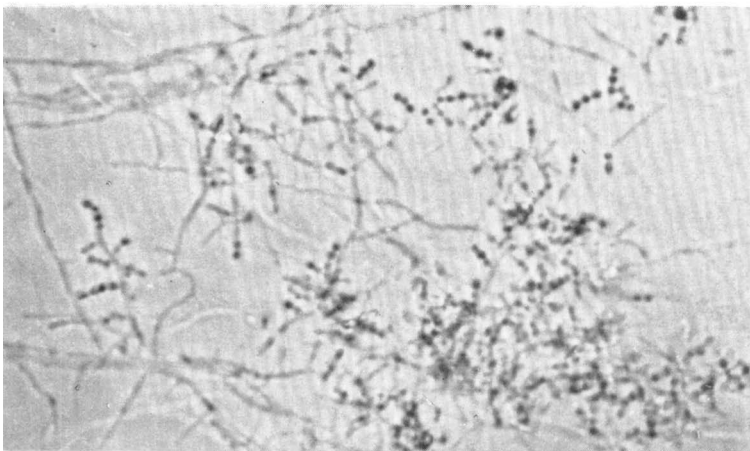
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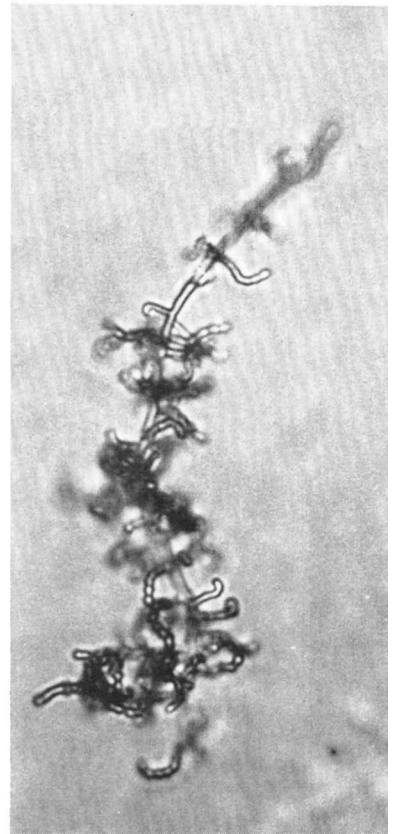
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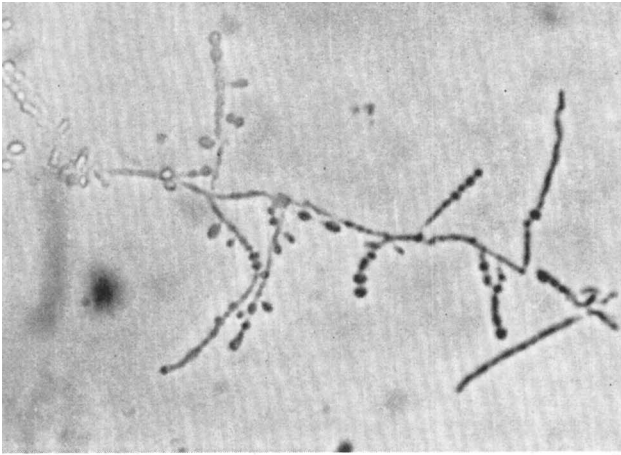
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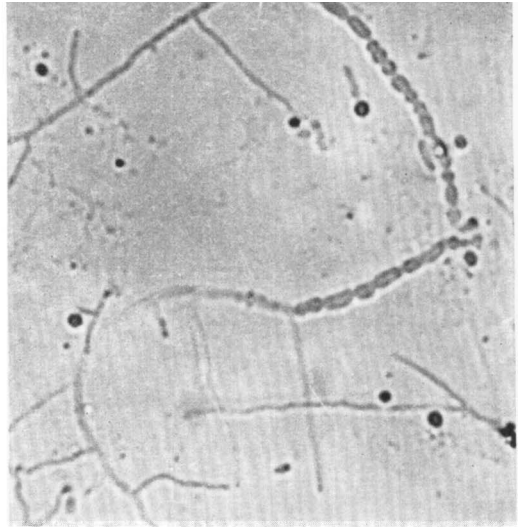
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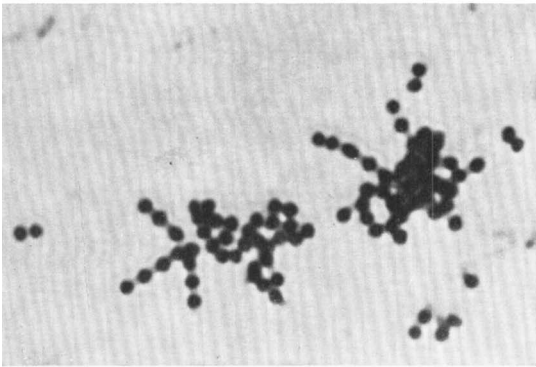
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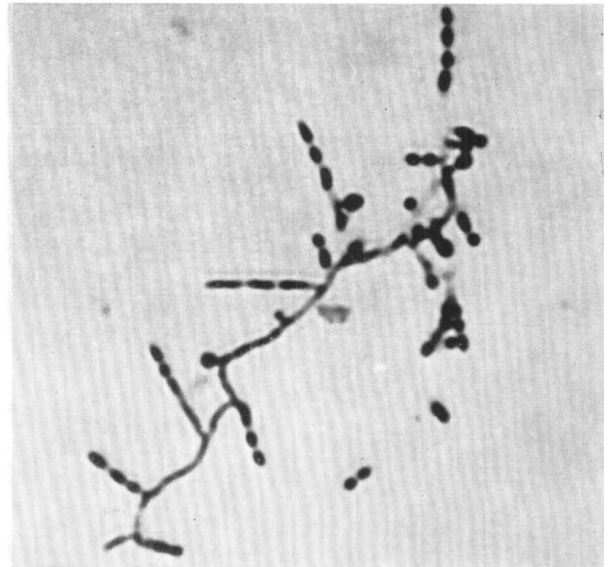
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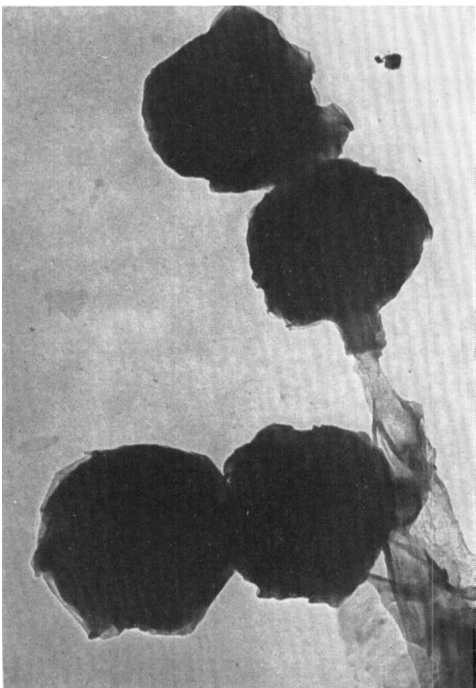
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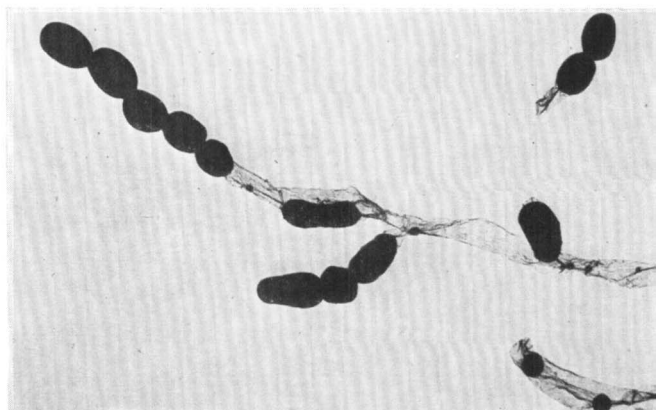
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## EXPLANATION OF PLATES

## PLATE 1

- Fig. 1. Aerial mycelium on potato dextrose agar *Micropolyspora faeni* CUB58 ( $\times 500$ ).
- Fig. 2. Aerial mycelium on yeast extract agar *M. faeni* CUB58 ( $\times 800$ ).
- Fig. 3. Substrate mycelium beneath surface of agar. Nutrient agar. *M. faeni* CUB58 ( $\times 850$ ).
- Fig. 4. Substrate mycelium beneath surface of agar. Yeast extract agar. Phase contrast *M. faeni* CUB58 ( $\times 800$ ).
- Fig. 5. Spore chains on surface of colony growing in slide culture. v-8 agar. *M. faeni* A94 ( $\times 980$ ).
- Fig. 6. Aerial mycelium of *Nocardia madurae* A443. Potato carrot agar ( $\times 800$ ).

## PLATE 2

- Fig. 7. Intercalary spore formation in slide culture. Nutrient agar. *M. faeni* A94 ( $\times 800$ ).
- Fig. 8. Intercalary spore formation in slide culture. Nutrient agar *M. faeni* A94 ( $\times 1600$ ).
- Fig. 9. Slide culture, dried and Gram stained. *M. faeni* A94 ( $\times 2000$ ).
- Fig. 10. Slide culture, dried and Gram stained *M. faeni* CUB58 ( $\times 2000$ ).
- Fig. 11. Electron micrograph of aerial mycelium spores *M. faeni* A445 ( $\times 7200$ ).
- Fig. 12. Electron micrograph of aerial mycelium spores. *M. faeni* A94 ( $\times 28,800$ ).