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SCANNING ELECTRON MICROSCOPY OF A SOIL FUNGUS GLIOCLADIUM ROSEUM

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Abstract

The paper describes the ultra-structural features of cryofixed and critical point-dried sporing cultures of a soil fungus Gliocladium roseum. Features described are Acrostalagmus-like conidiophores with spore masses in mucoid balls borne at the tips of branchlets and penicillate heads with conidia massed in rope-like formations. A particular feature of the aerial hyphae and conidiophores is the presence of blister-like swellings on their walls. These observations help to accurately characterize the organism in taxonomic studies.

Key Words: Scanning electron microscopy, Gliocladium roseum, cryofixation, low temperature Scanning electron microscopy, conidia

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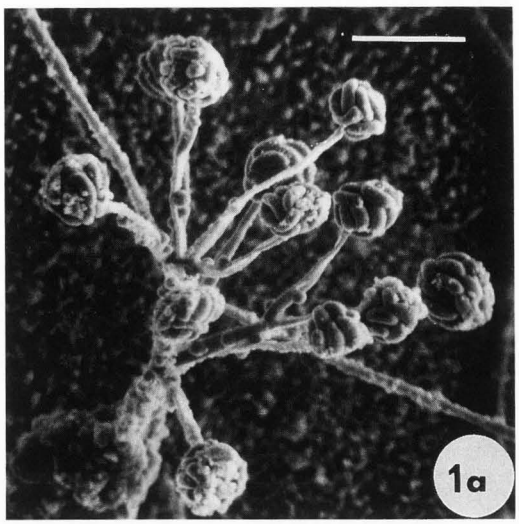
Introduction

We have frequently isolated the fungus Gliocladium roseum (Link) Bainier from agricultural soils during our studies on the microbiological degradation of phytotoxic phenolic acids. Its potential for biocontrol of the plant pathogenic fungus Verticillium dahliae has been reported by Keinath et al. (1991). Brian et al. (1951) described a member of the Gliocladium roseum series, isolated from a pine plantation soil in Dorset, England in 1948 and reported three new antibiotics from this species. In their studies, two types of conidial structure were noted from light microscope observations. In young colonies, the structure was a definite penicillus whereas in older colonies, the more common conidial apparatus was described as Acrostalagmus-like. The perfect stage of Gliocladium roseum has been described by Smalley and Hansen (1957) and the nomenclature discussed by Raper and Thom (1949). The present paper reports on the ultrastructural features of our isolate of G. roseum from scanning electron microscope studies.

Materials and Methods

The fungus was isolated on Oxoid Czapek Dox Agar CM97 at 25°C. Agar blocks supporting growth of the fungus were cryofixed and examined in a Cambridge Instruments S4 scanning electron microscope (SEM) with a Hexland cryo-system attached. Details of the technique have been fully described (Jones and McHardy, 1985; Jones et al., 1987) but a brief account of the method will be given here. The Hexland system consists of three components: the prechamber, the microscope cold stage and the decontaminator. The specimen was mounted in Leit-C, a conducting carbon cement, on an aluminium stub then was rapidly frozen in liquid nitrogen slush at -210°C (63K). The stub was then transferred to the pre-chamber, also cooled with liquid nitrogen. where it was sputter-coated with gold. The specimen was then examined on the cold stage in the SEM at approximately -150°C (123K). Occasionally, any ice that may have been present on the uncoated sample was sublimated by warming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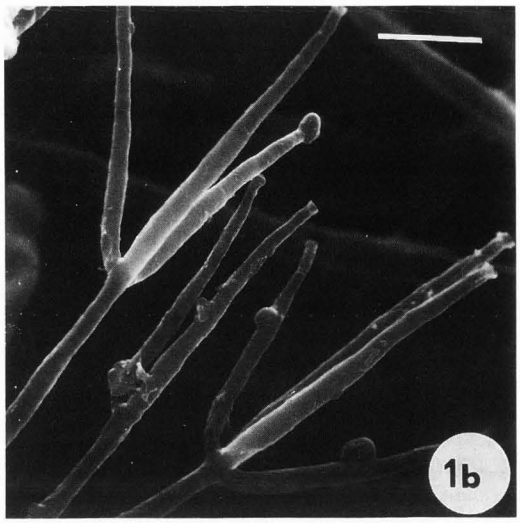
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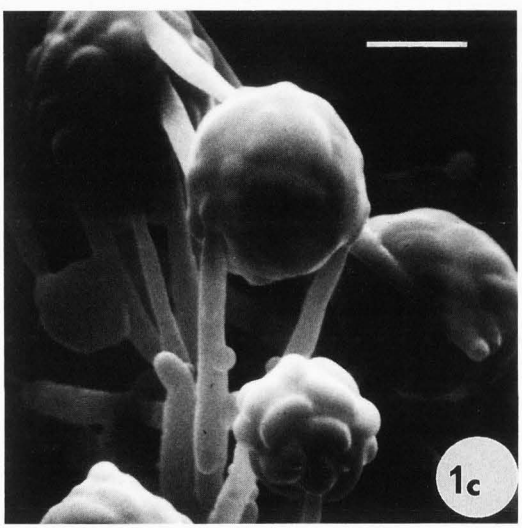
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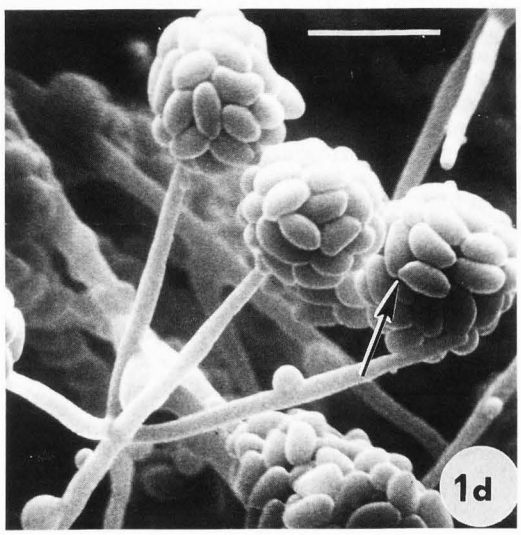
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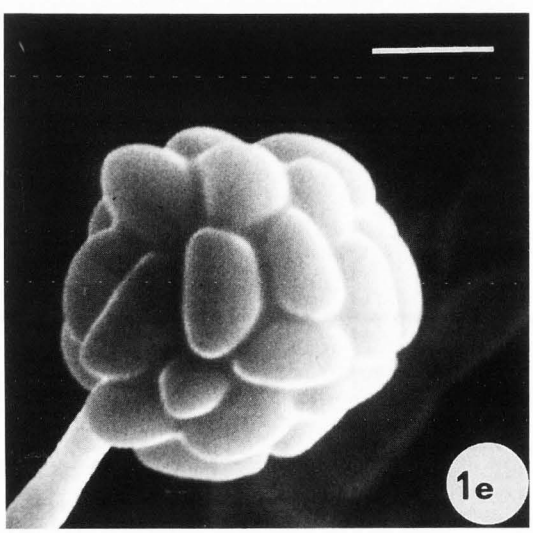
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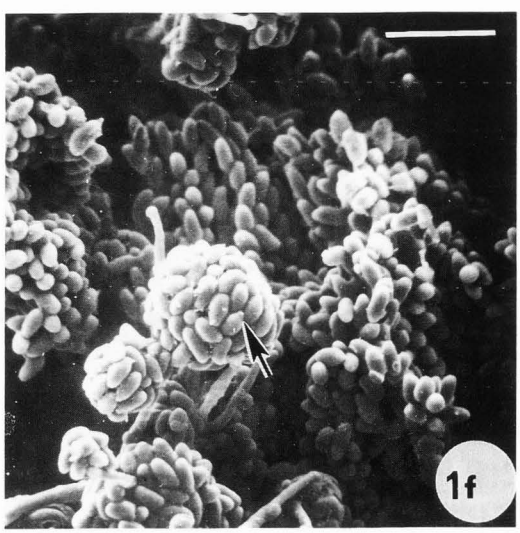
1f

the SEM stage to about -80°C (193K) for a few minutes. The SEM stage was then quickly cooled to about -140°C (133K) and the specimen sputter coated with gold.

For comparison, specimens from the same agar plates as above were also chemically fixed and critical point-dried (Jones, 1978a). Blocks of agar with sporulating structures were fixed in 3% glutaraldehyde in phosphate buffer for 1½ hours, followed by 2% osmium tetroxide in buffer for 1 hour at 4°C and 18 hours at room temperature, before being dehydrated in an ethyl alcohol series (30%, 75%, 90%, 100%, 15 minutes in each of the first three, 30 minutes in the last concentration). Specimens were then passed through a Freon 113/ethyl alcohol series (1:4, 2:3, 3:2, 4:1, 15 minutes in each), followed by Freon 113 until ready to critical point-dry from carbon dioxide in a Polaron apparatus. The blocks were fixed to SEM stubs with Leit-C cement and subsequently sputter-coated with gold.

Results and Discussion

The two types of conidial structure noted by Brian et al. (1951) were clearly distinguishable in cryo-fixed specimens. The Acrostalagmus-like conidial apparatus (or Verticillium phase referred to by Smalley and Hansen, 1957) consists of erect conidiophores branched in whorls with conidia borne at the tips of the branchlets in mucoid balls (Fig. 1a). Critical point-dried (CPD) specimens (Fig. 1b) reveal the conidiophore whorls but very rarely are groups of conidia at the tips retained. In etched specimens, sometimes only the spore outlines were seen because of the enveloping mucous (Fig. 1c) but in others the spores were clearly observed as reniform (kidney-shaped) to elliptical in shape (Fig. 1d). Fig. 1e is a higher magnification of a spore cluster with evidence of the mucous. The rarely seen clusters of spores at the tips of whorls of Acrostalagmus-like conidiophores in CPD specimens are shown in Fig. 1f; the spores terminate in short abruptly-pointed tips (apiculate), a feature sometimes seen in frozen hydrated material (Fig. 1d). The aerial hyphae and conidiophores have characteristic globose blister-like swellings on their surfaces which presumably give the roughened appearance noted in various species of the genus Gliocladium by researchers using optical microscopes. These swellings are present in frozen-hydrated specimens both before (Fig. 2a) and after (Fig. 2b) sublimation of ice that may be present on the specimen. These swellings were also conspicuous in CPD material and Fig.

Fig.1a. Acrostalagmus-like conidial apparatus. Conidia are in groups at tips of branchlets. Frozen-hydrated. Bar = 20 µm
Fig.1b. As Fig. 1a but critical point-dried. Note absence of conidial masses at tips. Bar = 10 µm
Fig.1c. Conidia, barely visible, in mucoid matrix. Frozen-hydrated. Bar = 10 µm
Fig.1d. Conidial clusters. Frozen-hydrated. Apiculate tips arrowed. Bar = 10 µm
Fig.1e. Conidial cluster. Frozen-hydrated. Bar = 5 µm
Fig.1f. Conidial cluster. Note apiculate tips (arrowed). Critical point-dried. Bar = 20 µm

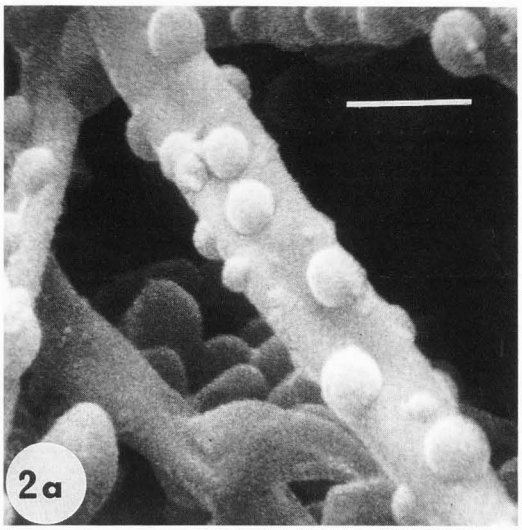

Fig.2a. Globose blister-like swellings on conidiophore branches. Frozen-hydrated. Bar = 5 µm

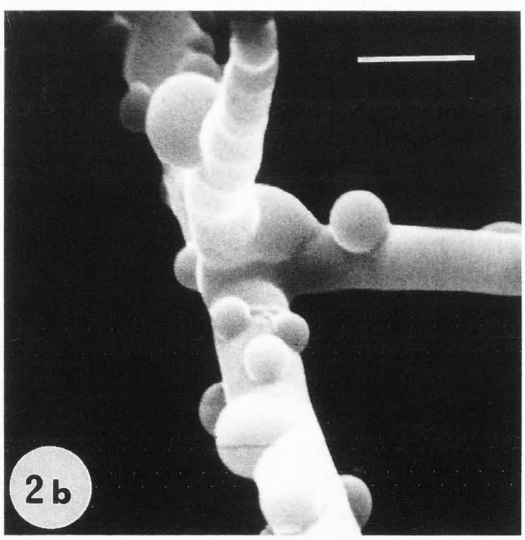

Fig.2b. Globose blister-like swellings on aerial hyphae. Frozen-hydrated. The temperature of these specimens had been raised to -80°C for 5 minutes then lowered again to -140°C prior to coating with gold. Bar = 5 µm

2c and Fig. 2d illustrate them in different aspects. Similar globose blister-like swellings have been noted previously in critical point-dried, freeze-dried and cryofixed setae of Volutella ciliata (Jones, 1980).

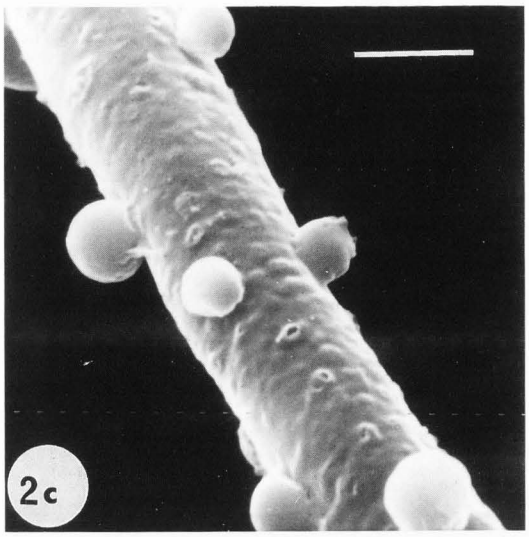

Fig.2c. Blister-like swellings on hyphal walls in critical point-dried material. Bar = 2 μm

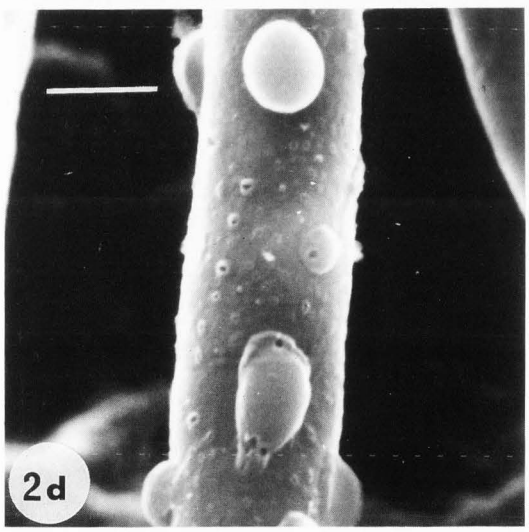

Fig.2d. Blister-like swellings on hyphal walls in critical point-dried material. Bar = 2 μm

Penicillate heads noted by Brian et al. (1951) were readily identifiable in cryofixed specimens (Fig. 3a,b) and the rope-like masses of conidia are striking features (Fig. 3c). Critical point-drying generally resulted in a distorted picture of the sporing masses and Fig. 3d is exceptional in that spore chains are preserved in their entirety.

The conidiophore structure is clearly quite variable, a point noted by Smalley and Hansen (1957) who referred to intermediates between the Verticillium and Gliocladium phases.

The sporulating structures described in this paper could not have been recorded accurately without using cryo-techniques which minimise the artefacts induced by conventional preparation methods such as dehydration and critical point-drying. The latter technique can result in shrinkage of structures and removal of solvent-soluble substances such as mucoid material (as shown in this paper) and waxes (Jones, 1978b). There is a strong superficial resemblance between Gliocladium spp. and Penicillium spp. but Gliocladium isolates 'produce conidia singly followed by enveloping in a ball of viscous slime rather than in chains' (Pitt, 1979). We confirm the presence of this mucous from our cryo studies here and this is important since the difference between Gliocladium and Penicillium is of fundamental taxonomic importance.

Acknowledgement

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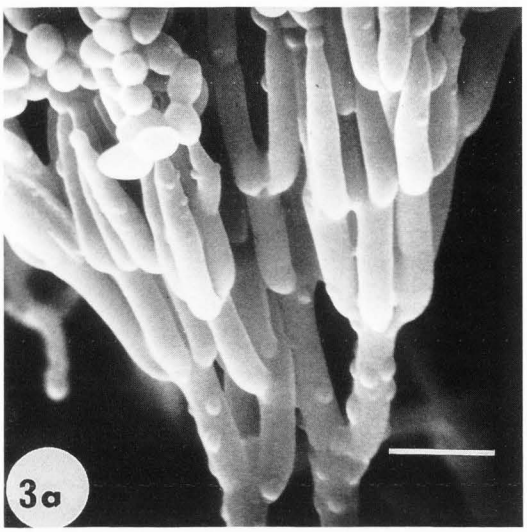
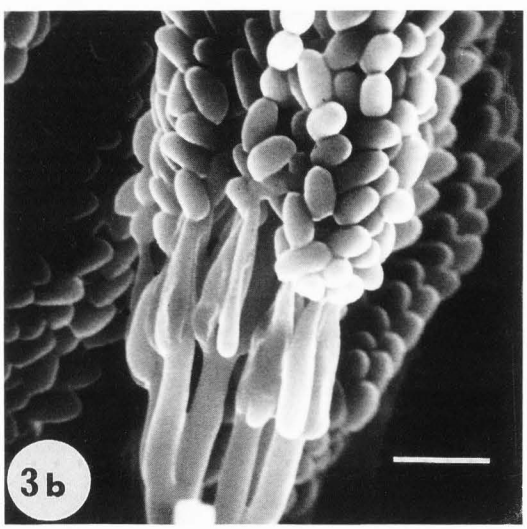
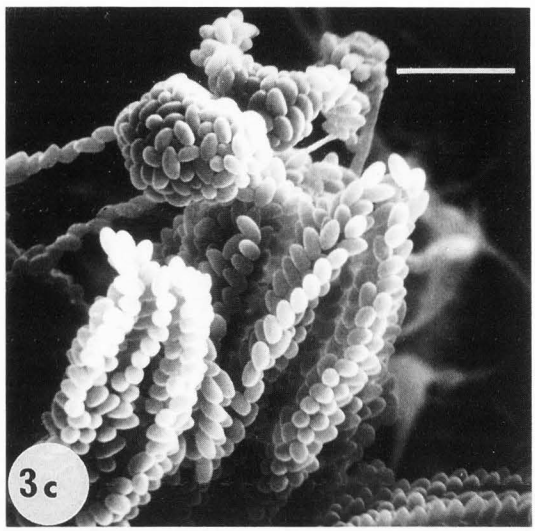




Figs.3a,b. Penicillate heads. Frozen-hydrated. Bar = 10 μm



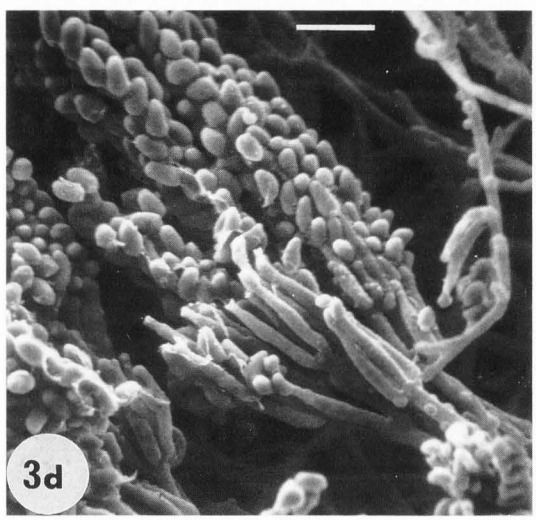


Fig.3c. Rope-like chains of conidia which arise from penicillate heads. Frozen-hydrated. Bar = 20 μm

Fig.3d. As Fig. 3c but critical point-dried. Bar = 10μm

Discussion with Reviewers

R.L. Grayson: What do the authors believe is the function of the blister-like swellings on the aerial hyphae surfaces?
Authors: Certain basidiomycetous fungi also exhibit outgrowths on their hyphae and the reasons for this phenomenon appear obscure.

R.L. Grayson: What might be the purpose of the small openings on the surface of the aerial hyphae? In fact are these true openings?
Authors: The pore-like features may be the remnants of a mucous that appears to coat the hyphae.

R.L. Grayson: How do you explain the formation of two different conidiophores and spore masses in Gliocladium roseum?
Authors: This morphology was noted by Brian et al. (1951) for a species of Gliocladium and by Smalley and Hansen (1957) and it is simply a feature of this particular fungus.

R.L. Grayson: There appears to be some evolutionary relationship between Gliocladium and Penicillium. What techniques would you suggest to gather evidence to support this view?
Authors: Difficult to offer suggestions. The fungus is unrelated to Penicillium but both have similar morphological characters with regard to the imperfect conidial stage.

R.L. Grayson: Why did you not give the perfect stage of Gliocladium roseum?
Authors: We have not seen the perfect stage in our cultures. Smalley and Hansen (1957) noted a perfect stage (perithecia) which developed along with conidial masses in their cultures of Gliocladium roseum.