SHORT COMMUNICATIONS

Demonstration of the Initial Cell in *Streptomyces griseus* by a New Microscopic Technique

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INTRODUCTION

The initial cell, arising upon the primary mycelium of *Streptomyces* and giving rise, in its turn, to the larger, sporogenous, secondary mycelium, was described by Klieneberger-Nobel (1947). Some confirmation was provided by Dickenson & Macdonald (1955), Saito & Ikeda (1959) and others, but it has also been claimed (Hopwood, 1960) that the secondary mycelium arises directly from the primary. An intermediate view was taken by Baldacci, Gilardi & Amici (1956) who observed forms resembling initial cells, but were reluctant to identify them as such because of the difficulties involved in the techniques then available. Recently, the problem has tended to be ignored by investigators of *Streptomyces* for the same reason: by ordinary microscopic methods, it is exceedingly difficult to observe the structure of anything beyond the extreme edge of a colony, whereas growth of these organisms on slides in fluid culture may be examined freely but does not truly correspond to the natural growth of *Streptomyces*. The technique described here was devised to overcome this problem by permitting parts of the interior of a colony, at various ages, to be examined directly under the microscope.

METHODS

Observations were made on a strain of *Streptomyces griseus* isolated in this laboratory. Cultures were grown in Petri dishes at 20 °C on nutrient agar, blood agar base (Oxoid) and Trypticase soy agar (Difco), diluted with non-nutrient agar (Oxoid) to concentrations 1:20 (v/v) of the standard.

The agar was cut away around suitable colonies at a distance of about 3 cm, and the trough was filled with 0.5 % methylene blue, which diffused through the agar to stain the colony dark blue. Pieces of agar, bearing colonies, were cut out and transferred to slides where they were permitted to dry partially and viewed without a coverslip.

Photomicrographs were made with an M40 × (Olympus) petrological objective and a PK 25 × (Zeiss Jena) ocular, illuminated by a quartz-halogen lamp, through an epifluorescence mirror (Zeiss, Oberkochen, West Germany) without the filters. With methylene blue-stained material, an orange filter was interposed between lamp and mirror. Where the depth of the structure was significantly greater than that of the focus of the microscope, photomicrographs were taken at different levels of focus and mosaics made to show different levels simultaneously (Fig. 1*a, b*).

RESULTS

The stained blocks of agar, bearing colonies, were opaque to transmitted light. By incident illumination, the agar was colourless or very pale orange. In contrast, the mycelium and spores appeared mainly bright blue. Contrast was increased by using an orange filter.

Bodies resembling initial cells could clearly be observed (Fig. 1). In relatively young cultures (about 2 to 4 weeks) the attachment of thinner primary and thicker secondary filaments could be recognized (Fig. 1*a, b, c*). In old cultures (about 3 to 4 months) the primary filaments had degenerated and were seen as paler lines on the surface of the medium. These passed into the initial cell, from which chains of spores protruded. The body was

Fig. 1. (a, b, c) Initial cells from a young colony (3 weeks) showing narrow primary and wider secondary filaments attached to a stained body; (a) and (b) are mosaics made from two prints at different levels of focus.

(d, e, f) Initial cells from older colonies (about 3 months). The primary filaments have degenerated, leaving light lines on the surface of the medium. The secondary mycelium consists mainly of chains of spores and some part of the central body may consist of a mass of spores. Bar marker represents 5 μm .

five to six times wider than the larger filaments, and darkly stained. There was no obvious appearance of 'nests', comprising interwoven primary filaments, as described by Klieneberger-Nobel (1947), but some of the bodies looked as if they might be partially composed of concreted spores (Fig. 1d, e, f).

DISCUSSION

The general nature of the bodies illustrated in this paper agrees with the concept of an initial cell arising from the primary filaments and becoming the source of the secondary sporogenous mycelium. Complex nests of primary filaments were not seen in this material. The technique has obvious applications to the further study of the problem since it provides an exceptionally clear visualization of these complex structures both *in situ* and undisturbed.

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