SHORT COMMUNICATION

A Method for the Examination of the Substrate Mycelium of Actinomycetes by Scanning Electron Microscopy

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The polyol gel Lutrol FC127 was used to solidify culture media. This gel liquefies as the temperature drops below a critical value for the concentration used. This property was used to recover whole colonies of Thermoactinomyces sp. for examination of the substrate mycelium by scanning electron microscopy.

INTRODUCTION

The study of actinomycete morphology and morphogenesis by scanning electron microscopy (SEM) has been repeatedly reported (Williams & Davies, 1967; Baldacci et al., 1971; Locci, 1971; Sharples & Williams, 1976). In order to examine the mycelium growing within a substrate by SEM it is first necessary to remove the substrate. Solid growth media could be liquefied and then displaced by washing with water or other liquid before preparing for examination. Since the usual solidifying agents liquefy at temperatures at which cellular damage would occur (e.g. agar liquefies at 85 °C), it is not possible to recover the substrate mycelium in an undamaged form by the use of temperature, unless a solidifying agent that liquefies at a lower temperature is used.

The use of the polyol gel Pluronic F127 as a solidifying agent for enrichment, isolation and growth media for denitrifying bacteria, sulphate reducers and methanogens was demonstrated by Gardener & Jones (1984). The polyol gel, which is relatively non-toxic, liquefies as the temperature drops below a critical value for the concentration used. This communication reports the use of an equivalent polyol gel (Lutrol FC127) as a solidifying agent for growth media which enables the removal and subsequent examination of substrate mycelium by SEM.

METHODS

Lutrol FC127 (Blagden Campbell Chemical, AMP House, Dingwall Road, Croydon, Surrey, UK) was dissolved in nutrient broth (1-3%, w/v) at a concentration of 20% (w/v) at 4 °C (Gardener & Jones, 1984). The medium was sterilized by autoclaving at 121 °C for 15 min and then cooled to 4 °C before dispensing. The medium was inoculated with a Thermoactinomyces sp., isolated from composted bark by the method of Cross (1968), and incubated at 50 °C for 3 d.

After incubation the cultures were cooled to 4 °C to liquefy the medium. The colonies were removed and fixed in 3% (v/v) glutaraldehyde in 0-1 M-potassium phosphate buffer (pH 6-8) for 16 h. The remaining polyol gel was removed by gentle agitation in the buffer for 6 h, with changes of buffer every hour. The colonies were then dehydrated in an ethanol series (20, 40, 60, 80, 95 and 100%, v/v; 15 min per concentration) and dried with hexamethyldisilazane (Nation, 1983). The above fixing, washing and dehydration procedures were done at 4 °C.

The dried samples were mounted on aluminium stubs with colloidal carbon, coated with gold and examined with a Cambridge S4 stereoscan.

Abbreviation: SEM, scanning electron microscopy.

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RESULTS AND DISCUSSION

Lutrol FC127 forms a semi-rigid gel which can be inoculated with a spore suspension. The appropriate concentration of the polyol is dependent on the incubation temperature to be used, being 20 to 30\% (w/v) for temperatures between 50 °C and 20 °C, respectively. The polyol, which liquefied at 4 °C, was completely removed during the fixation, washing and dehydration stages, as can be seen in Fig. 1.

This method enables the mycelium and structures produced in the solid growth media to be studied, unlike the previous studies of the growth and micromorphology of actinomycetes, which have concentrated on the mycelial development on surfaces such as dialysis tubing (Afrikian et al., 1973), coverslips and slides (Williams & Davies, 1967; Locci, 1976) and the growth media (Williams, 1970). Fig. 1 shows the interior of a colony with the mycelium from the surface developing down.

Thermoactinomyces spp. produce endospores (Cross, 1970) on both aerial and substrate mycelia (Goodfellow & Cross, 1983). The endospores can be observed by light microscopy, but only those produced above or on the substrate surface have been studied by SEM (Cross et al., 1971; Williams et al., 1973). Fig. 2 shows the endospores within the substrate mycelium. Endospores released by the autolysis of the parent hyphae (Williams et al., 1973) were not observed.

The ability to remove colonies from a solid growth medium could enable growth kinetics to be studied, since both the total hyphal length and number of tips could be determined on the colonies recovered from the medium; previous studies have been limited to microculture techniques (Schumann & Bergter, 1976; Kretschmer, 1978) and chemostat cultures (Kretschmer et al., 1981). Furthermore, this method could also be used to recover whole colonies for chemical analysis to study nutrient movement from the medium into the mycelium.
REFERENCES


