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A technique for replica plating *Coprinus lagopus*, a filamentous fungus

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SUMMARY

The problem of replica plating filamentous fungi such as *Coprinus lagopus* is overcome by inducing micro-colonies with sodium deoxycholate and using 'Velcro', a hooked material, to replace velveteen in the standard replica plating technique. 'Velcro' is advantageous in that it has a regular pattern of closely spaced hooks which transfer small inocula from the colonies on the master plate.

1. INTRODUCTION

Adaptations of Lederberg's replica plating technique (Lederberg & Lederberg, 1952), originally designed for bacteria, have been successful with certain fungi. Mackintosh & Pritchard (1963) found that with the induction of micro-colonies with sodium deoxycholate and the use of damp velveteen they were able to replica plate *Aspergillus nidulans*. This method is successful with fungi that produce copious dry spores. Other methods have also proved effective – the use of a perspex block embedded with steel pins (Roberts, 1959) or filter paper (Maling, 1960), along with morphological mutants of *Neurospora crassa*, which produced restricted growth.

However, all these methods fail with *Coprinus lagopus*, a basidiomycete fungus. Colonial morphology of *C. lagopus* consists of a mass of aerial mycelium with the oidia, often in abundance, firmly enmeshed in the mycelium and so failing to adhere to damp or dry velveteen.

This note describes a method which could be useful in the replica plating of filamentous fungi by inducing micro-colonies with sodium deoxycholate and overcoming the problem of spore attachment by using 'Velcro', a hooked material which transfers small inocula of mycelium.

2. MATERIALS AND METHODS

Two strains of *Coprinus lagopus* were used, H5, a wild-type strain and NG184, *ad8*[2] auxotroph (supplied by L. Casselton). Standard culturing techniques and media as described by Lewis (1961) were used.

Culture plates were scraped and an oidial suspension was made in sterile distilled water. Appropriate concentrations of oidia were plated on to complete medium (CM) supplemented with 0·015% sodium deoxycholate obtained from British Drug Houses Ltd. Oidia were spread with a glass rod. Plates were incubated at 37° for 48 h.

The material used for plating was 'Velcro' (supplied by Selectus Ltd., Biddulph, Stoke on Trent, ST8 7RH, England). 'Velcro' has two adhering components; only the

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rough, hooked side was used and this can be purchased separately. The maximum width available was 2 in. and it was necessary to sew two pieces together giving a final width of 10 cm, cut into 12 cm long strips. Strips of 'Velcro' were autoclaved in aluminium foil. It was possible to use the strips innumerable times. Sterile strips were held in position on an 8 cm diameter perspex cylinder by a metal ring. Master and replica plates were inverted and pressed on to the 'Velcro'. Best results were obtained by using a concentration of 0·01 % sodium deoxycholate in the replica plates. This slightly lower concentration in the replica plates produced optimum growth – the higher concentration used in the master plates inhibited growth. Whereas the master plates were incubated for 48 h, it was only necessary to incubate the replica plates for 24 h. The reasons for this were firstly the lower concentration of sodium deoxycholate in the replica plates did not retard growth to such an extent and secondly, the fact that in the master plates one had to allow for the lag phase of oidial germination. Transference to the replica plate, however, consisted of mycelial inocula which could grow without a lag phase. The response expected is all or none in the case of auxotrophs and 24 h proved adequate time for colonies to establish themselves. It was essential that all plates should have a thick layer of agar to prevent the agar being ripped by the 'Velcro'.

3. RESULTS

As with *Neurospora* and *Syncephalestrum* (Tatum, Barratt & Cutter, 1949) and *Aspergillus* (Mackintosh & Pritchard, 1963), sodium deoxycholate induced microcolonies in *Coprinus*, making it possible to obtain replicas of a maximum of 100 colonies per plate, reducing the colony size to 3–4 mm and producing a distinct edge. There was a variation in colonial size due to the delayed germination of a percentage of oidia under normal or sodium deoxycholate treatment. Sodium deoxycholate seemed to have no effect on oidial production and only slightly reduced viability.

Table 1. *Results of the reconstruction experiment to test the efficiency of the replica plating technique*

Individual viabilities	
NG 184	8 %
H 5	12 %
Expected combined viability	10 %

Strains	Actual viability	Total no. of oidia on 3 master plates	Total viable oidia	Scored by replica plating	NG 184 *ad8*[2] Estimated no. plated
NG 184 + H 5	9	1095	99	37	39

The advantage of 'Velcro' was that it actually nicked out tiny pieces of agar with mycelial fragments and so the problem of oidial attachment was overcome. One could imagine this method being adapted for the selection of aconidial mutants. The hooks are arranged in a very regular pattern 1 mm apart, so overcoming the problem of the transfer of very small colonies. The join down the centre of the two strips provided an orientation line which proved useful.

All the colonies within the border of the 'Velcro' were transferred from the master plate. Those outside the 'Velcro' were not transferred but this could be overcome by restricted spreading. Using this method up to four replicas of the master plate could be made.

A reconstruction experiment to test the efficiency of the system was performed. Equal concentrations of H5 and NG184 (*ad*-8^2) were plated on to CM plates supplemented with 0·015 % sodium deoxycholate, to give a final total number of oidia per plate, within the range possible for this technique. Individual viabilities of H5 and NG184 were established by testing them separately on the same medium. Replicas of the combined master plates were made on minimal medium (MM) and then CM, both supplemented with 0·01 % sodium deoxycholate. The second replica plate on CM provided a check for the *ad*$^-$ colonies that did not grow on the minimal replica plate. Colonies that did not grow on the minimal medium plate were traced to the complete replica plate and tested on minimal medium to establish if they were true *ad*$^-$ colonies. The results of the experiment are given in Table 1. The recovery from the replica plates ranged between 95 % and 100 %.

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