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With 1 *plate*
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The production and replica plating of micro-colonies of *Aspergillus nidulans*

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1. INTRODUCTION

The value of replica-plating with velveteen (Lederberg & Lederberg, 1952) in microbiology is well known. Unfortunately the technique in its original form cannot usually be used for replicating fungi with dry spores since these tend to get splashed when the velveteen is applied. One way to avoid this difficulty is to use a Perspex block embedded with large numbers of steel pins instead of velveteen (Roberts, 1959). In the particular case of *Aspergillus nidulans*, a further limitation to the technique is the rather small number of colonies which can be handled on one plate without growth becoming confluent. We find that 150 is the upper limit in practice.

A method is reported here for obtaining micro-colonies of *A. nidulans* about 3 mm. in diameter, thus overcoming the second limitation described above, together with a simple method for obtaining replicas of plates containing up to 300 micro-colonies.

2. MATERIALS

Four strains were used: *bi1 meth1*, *bi1 w3*, *pro1 y Acr1*, and *paba1 y2 pyro4*. All were from the culture collection of the Department of Genetics, University of Glasgow. Sodium desoxycholate (SD) was obtained from British Drug Houses Ltd. Velveteen was supplied by J. Pallu and Lake Ltd.

3. METHODS

The cultural techniques employed were those routinely used for *A. nidulans* (Pontecorvo *et al.*, 1953) with two exceptions. First, the solid complete medium (CA) used had the following composition: Difco-Bacto peptone, 1·0 g.; Difco Casamino acids, 1·0 g.; Difco yeast extract, 1·0 g.; adenine, 0·15 g.; vitamin solution (Pontecorvo *et al.*, 1953), 10 ml.; glucose, 10 g.; Difco-Bacto agar, 15 g.; distilled water, 1000 ml. pH was adjusted to 6·0. Secondly, platings of conidia and ascospores were made by adding an appropriate aliquot of a spore suspension to 3 ml. of melted soft agar (0·6% Difco agar in water) held at 46° in a water bath, and adding this as a top layer to previously poured plates. This method gives a more even distribution of spores over the plate than does spreading with a glass rod.

Velveteen discs (12 cm. diameter) for replica plating were washed and autoclaved in petri-dishes of similar size while still damp. A sterile disc was placed on a cylindrical wooden block (diameter 8 cm.) and held in place with a brass ring. The master plate and the replica plates were inverted and lowered on to the velveteen. Replica plates were scored after incubation for 24 h. at 37°. This procedure is that used routinely in this

laboratory for replica plating of bacteria with the important exception that the velveteen was used damp.

4. RESULTS

(i) *Formation of micro-colonies*

Plating conditions were first looked for which would lead to the formation of micro-colonies and thereby permit the use of higher plating densities than is normally possible. At the same time it was clear that success in this direction would make Robert's (1959) method of replica plating less satisfactory since the spacing between the pins on his replicator (5 mm.) would limit the reduction in colony diameter that could be employed.

Acting on a report (Van Arkle, 1958) that an alkyl aryl sulphonate induced compact growth in *A. nidulans*, a number of surface-active agents were screened for their effect on colony morphology. A suitable compound would require not only to induce colonies of restricted size but also produce no inhibition of conidium production and have no effect on viability. Only two compounds (sodium docecyl sulphate and sodium desoxycholate (SD)) had all of the properties looked for and the effect of SD was investigated in more detail.

Colony diameter on minimal agar (MA) was approximately proportional to the concentration of SD in the medium but was also affected by the number of colonies per plate. Figure 1 shows the effect of a final concentration of 0·08% which was found to be most satisfactory. It was necessary to add SD to both the top and bottom agar when using the overlay method in order to get satisfactory results. Formation of conidia appeared to be more profuse in the presence of SD than in its absence on MA, but on CA the reverse was found. Inhibition of conidiation on CA was not serious enough, however, to interfere with subsequent replication. In preliminary trials SD at the concentrations used to induce colonial growth did not affect viability of either ascospores or conidia and did not appear to be mutagenic. Cursory microscopic examination of colonies growing on MA with SD suggested that the morphology of hyphae at the margin of micro-colonies was normal.

No attempt was made to elucidate the mechanism of action of SD. There is some indication, however, that the effect is not simply due to a reduction of surface tension of the medium. After this work was completed we learned that SD had already been shown to induce colonial growth of *Neurospora* and *Syncephalastrum* by Tatum *et al.* (1949). These authors showed that only anionic detergents induced colonial growth in these organisms. Our experiments so far as they go suggest that the same is true for *A. nidulans*. Thus Tween 80 (non-ionic) and Roccal (cationic) did not induce compact growth at concentrations which were not lethal, whereas sodium dodecyl sulphate (anionic) behaved like SD.

(ii) *Replica plating*

Micro-colonies can be replica plated very successfully (Fig. 2) provided two conditions are observed. The velveteen should be damp and the pile should be as short as possible. Using velveteen pads prepared as described under Methods, up to eight replicas can be taken from a single master plate without serious loss of resolution. Table 1 shows the result of a reconstruction experiment in which plates containing an average of seventeen colonies of a strain requiring proline for growth and increasing numbers of colonies of a proline independent strain were replica plated on to MA without proline. Both strains had the same conidium colour and were morphologically indistinguishable in order to avoid subjective bias in scoring. At a total colony density of 150 per plate the majority of the proline-requiring colonies were identified. At higher colony densities there was some reduction in the efficiency of recovery, although in more recent experiments we have

obtained almost full recovery with 300 colonies per plate. This method of replica plating therefore compares favourably in efficiency with that of Roberts.

Table 1. *Efficiency of detection of an auxotroph by replica plating*

No. of plates	*pro*+ and *pro*− colonies		*pro*− colonies		
	Per plate	Total	Scored by replication	Estimated no. present	Estimated recovered (%)
5	69	344	86	85	100
4	93	377	63	68	93
3	133	400	42	51	82
3	282	845	30	51	59

A constant number (*c.* 17) of conidia from the strain *pro1 y Acr1* and increasing numbers of conidia from the strain *paba1 y pyro4* were plated on MA + proline, *p*-aminobenzoic acid and pyridoxin and replicated on to MA without proline.

5. SUMMARY

Micro-colonial growth on semi-solid media is induced in *Aspergillus nidulans* by sodium desoxycholate. A method is described for replica plating micro-colonies with damp velveteen.

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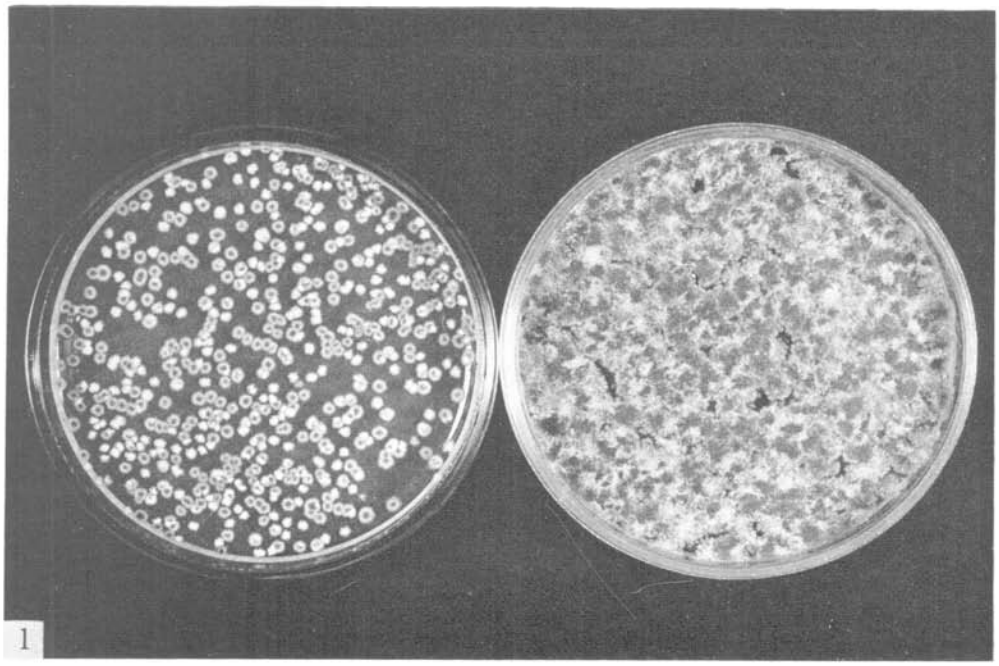

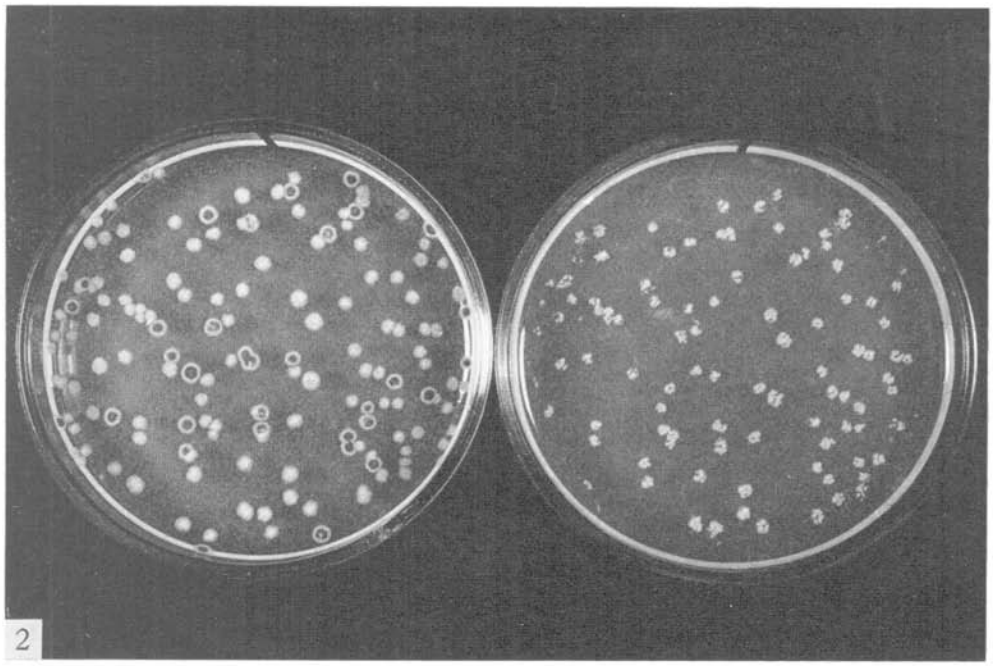

Fig. 1. The effect of SD on growth of *A. nidulans*. A mixed suspension of conidia from a white and a green strain was plated to give about 600 colonies per plate on MA with SD (*left*) and without SD (*right*). The plates were incubated for 48 hr.

Fig. 2. Replica plating with damp velveteen. The plate on the left contains colonies of two types, green methionine dependent and white methionine independent. The plate on the right is a replica of that on the left on MA without methionine. Both plates contain SD.

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