Synchronous induction and development of ascogonia

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Abstract
Synchronous induction of ascogonia

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Of the several kinds of differentiated cells produced by euascomycetes such as *N. crassa* the ascogonium is perhaps the most singular. And yet, this coil of cells has rarely been described, much less studied, in this species (see review by G. Turian, 1978 in *Vol. III The Filamentous Fungi*, eds. Smith and Berry). A reason for this relative neglect may simply be technical difficulties. Under the usual cultural conditions these coils appear rather late in the development of a colony, often under or in a dense mat of vegetative hyphae and conidiophores, and over a time period of several days.

To overcome these difficulties the following technique was devised. The first step is to reduce the density of growth in the colony through the use of a water-2% agar medium. Next, once the colony has matured (45 days), a nutrient supplement is added locally to certain areas of the colony. This results in a localized proliferation of hyphae that differentiate ascogonia more or less synchronously.

The details of the technique are as follows: A wild-type strain of either mating-type is inoculated centrally on a layer of the water-2% agar medium (25 ml) in a 9 cm petri plate. During 4-5 days of incubation at 25°C the colony produces mostly some widely scattered protoperithecia (Bistis, 1981 *Mycologia* 73: 959-975). If now 1-4 blocks (5x5x0.6 mn) of SC agar medium (the nutrient supplement) are placed directly on the surface of the colony (1 cm from the edge), a localized proliferation of new, narrow-gauge hyphae occurs within 24 hr. These will grow around, within and on the surface of the individual agar blocks. Within the next 6 hr several dozen ascogonia will develop as branches of the hyphae that are on the surface of the block. Also, since this ascogonium producing hyphal system is relatively sparse and limited to the surface (no aerial branches), the view is unobstructed.  

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Feher, Zs. and M. Schablik

Fast preparation of *Neurospora* DNA for Southern analysis.

We propose two methods for preparation of DNA from small amounts of *Neurospora* crassa mycelium. Both methods were described for yeast (Struhl et al. 1979 *Proc. Nat. Acad. Sci. USA* 76: 1035-1039, Kingsman et al. 1979 *Gene* 7: 141-151) and were adapted for *Neurospora* with minor modifications.

Mycelia for DNA preparation were grown either on cellophane-agar plates (Schablik et al. this issue) or in 100 ml liquid overnight (16-18 hours old) cultures.

Protoplast formation was carried out in 1 M sorbitol, 10 mM MOPS (pH 5.8) containing 1% Helicase L’Industrie Biologique Francaise) and 1% Chitinase (from *Serratia marcescens*, SERVA) with gentle shaking at 27.29°C for 2 hours (Schablik et al. this issue). Spheroplasts or osmotically unstable hyphal fragments can be obtained with the same treatment from submerged culture, as well. The protoplasts (or spheroplasts) were washed three times with 1 M sorbitol, 25 mM EDTA pH 8.0 solution to remove nuclease contamination. Cells from either one cellophane-agar plate or one flask were collected in an Eppendorf tube and further processed according to one of the following methods:

1. Cells were suspended in 500 µl 50 mM EDTA, pH 8.0, 0.3% SDS solution. Then, 2 µl diethyl pyrocarbonate was added. The mixture was incubated at 65°C for 10 min and cooled on ice. After addition of 100 µl 5 M K acetate the precipitated proteins were centrifuged in an Eppendorf centrifuge for 5 min. The nucleic acids were precipitated from the supernatant by 2 volumes of ethanol, collected by centrifugation and dissolved in 40-50 µl of 10 mM Tris pH 8.0, 10 mM NaCl, 1 mM EDTA. RNA was removed by adding pancreatic RNase to a final concentration of 25 µg/ml and incubating at 37°C for 1 hour.

2. Cells were suspended in 500 µl 50 mM Tris pH 8.0, 5 mM EDTA solution and 25 µl 20% SDS was added. After 10 min incubation at room temperature 125 µl 5 M NaCl was added. The lysate was incubated on ice for 1 hour then centrifuged for 5 min in an Eppendorf centrifuge. RNase was added to a final concentration of 25 µg/ml and the mixture was incubated at 37°C for 1 hour. Then it was extracted with 0.5 vol phenol, 0.5 vol chloroform and twice with an equal volume of chloroform. DNA was precipitated with 2 volumes of ethanol, collected by centrifugation and dissolved in 40-50 µl 10 mM Tris, pH 8.0, 10 mM NaCl; 1 mM EDTA.

Both protocols are suitable for the parallel preparation of DNA from several independent cultures. The purification can be carried out throughout in Eppendorf tubes of volumes 1.5 or 2 ml. The DNA provided by these methods is pure enough to be digested by restriction endonucleases and to be used in Southern type experiments. 20-30 µg DNA can be obtained from one cellophane-agar plate or from 100 ml liquid overnight culture. This amount of DNA is sufficient for several digestions. (Supported by the Hungarian Academy of Sciences.) Institute of Biology, University Medical School, H-4012 Debrecen, Hungary.