Method for isolating protoperithecia

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Abstract
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A protoperithecia-less (ft-1) mutant (Tan and Ha 1970 Mol. Gen. Genet. 107: 158) also does not develop protoperithecia under these conditions. Several other mutants defective in protoperithecial development have also been cultured. For several of these, the aerial hyphae growing on the vertical walls of the dish are shorter than those of wild type. (This work was carried out under a Gosney Fellowship at the laboratory of N. H. Horowitz, Division of Biology, California Institute of Technology) Division of Genetics, School of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia.


It is frequently laborious to examine the segregation of isozymes which are recognized by electrophoretic criteria. The problem is aggravated when recombinants of closely linked genes are being sought, as is the case with the genes for trehalase in N. crassa. In this example recombinants were being sought between the tre gene (coding for the presence of trehalase) and the mig gene (coding for the electrophoretic mobility of the enzyme, viz. fast or slow) which mapped less than one unit apart (Sussman et al. 1971 J. Bacteriol. 108: 59). An enrichment of recombinants was achieved in the usual way through the use of flanking markers, and the identification of “fast” and “slow” forms of trehalase in tre+ recombinants would normally have involved electrophoresis polyacrylamide gel. However, the use of an immunological technique made it possible to screen up to 100 ascospore isolates Per day with a high degree of specificity. The technique is described here since it may have general application in Neurospora studier.

Numerous methods have been employed for the production of antibodies and generally require up to 10 mg of pure antigen as starting material. In the case of trehalase, 2-10 mg of pure enzyme in 0.05 M phosphate buffer (pH 5.6) was emulsified with an equal volume of Freund’s adjuvant and injected intra-muscularly into rabbits over a period of 2 weeks. Booster injections contained approximately 1 mg of antigen in incomplete adjuvant. A maximum titre was obtained in the third week and the animals were then bled by cardiac Puncture or from an ear vein. The 20-30 ml of blood obtained was allowed to clot in a Petri dish and, after standing overnight at 4°C, the serum which had separated was decanted, centrifuged for 20 min at 3,000 x g to remove cells, and stored at -20°C.

The Ouchterlony double diffusion method was used to screen extracts. Degraeted glass slides (2 in. x 2 in.) were first dipped in 0.25% agar#2 (in deionized water), drained and permitted to dry. 2 ml of 1% agar#2 (in 0.04 M veronal/HC1 buffer, pH 7.5, + 0.003% methyl orange) war then poured to form an even layer on one side of each slide. After storage at 4°C for 12 hr, one central and four peripheral wells were cut in each slide wing a template and cork-borer. The antiseraum was placed in the central well and peripheral wells contained extracts of undesigned phenotypes alternating with sampler of the electrophoretically identified isozymes “fast” or “slow”. The extracts were prepared from acetone powdered mycelium as described previously for electrophoresis (Yue et al. 1971 Genetics 68: 473) and 10 µl samples of antisera and antigens were used throughout.

Clear-cut differences between extracts of known “fast” and “slow” strains were obtained using antisera prepared against trehalase from the wall fraction of “fast” strains, and such differences were annulled by absorption of the antiseraum with purified “fast” trehalase. A total of 574 tre recombinants were screened by this method and only one yielded on ambiguous result on Ouchterlony plates. Electrophoresis was used to check 10% of the samples selected at random and in all cases the expected result was obtained.

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In this method, protoperithecia are grown for seven days at room temperature on petri plate containing 15 ml of Westergaard’s crossing medium with 1.5% agar. After this time, large numbers of protoperithecia are visible. The agar from one plate is cut into squares, and is then immersed in 50 ml of sterile water and ground in a Sorvall Omnimixer at setting nine for one minute. This suspension is diluted into 2 liters of water and stirred continually at room temperature during the rest of the procedure. It is important that this suspension be dilute and that clumps of mycelia not be allowed to form.

The suspension is siphoned at a rate of 300 ml/hr into a race tube filled with distilled water. Both ends of this U-shaped tube have been bent about 30° from the horizontal. The bent portion at one end is slightly longer so that the suspension will flow through the tube and out the lower end without pumping. Most of the mycelial material either flows completely through the tube or collects near the far end, while the protoperithecia tend to collect near the mouth of the tube.

We collect the material found in the first ten cm of the tube and find it to be relatively free of mycelia. A second passage of this material giver protoperithecia almost free of any contaminating mycelia. We have been able to collect as much as one gram (wet wt.) of protoperithecia from four petri plates. The protoperithecia seems to have suffered badly from the isolation procedure, as most of them can still serve as foci for vegetative growth and form a distinctive colony type when plated on sorbose media. We have been unable to fertilize these isolated protoperithecia, possibly because the trichogynes are removed during the initial process of shearing. We are currently characterizing some of the proteins which are contained in the protoperithecia. Department of Genetics, University of Washington, Seattle, Washington 98105.