A rapid extraction method for mycelial organelles

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Abstract
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(1891–1893) with the following modifications: using a 50 μ Hamilton syringe, the capillary tube is filled with separating gel solution to a depth of 0.5 cm, and proportionately twice as much stacking gel is used. Pieces of cellulose acetate film (Sephaphore III, Gelman) cut to fit into the capillary tube, are washed with 0.1 M phosphate buffer, pH 7.0. A single perithecium is quashed onto a cellulose acetate piece. If desired, microscopic analysis of ascus and ascospore morphology can be performed before the cellulose acetate piece is placed on the surface of the stacking gel. A small volume of stacking gel solution is then layered on top of the cellulose acetate piece to prevent diffusion of the proteins back into the electrophoresis buffer. The upper part of the capillary tube is filled with electrophoresis buffer containing Bromphenol Blue as tracking dye. Electrophoresis is run in a standard Tris-citrate electrophoresis apparatus, at 1/2 ma per gel. After the tracking dye has migrated lcm down the separating gel, the current is stopped and the capillary tubes are immediately submerged in ice both to delay band diffusion. The gels are removed from the capillary tube using a syringe filled with ethylene glycol and provided with a thin needle (No. 27 or 30). The gels are fixed in 10% (w/v) trichloroacetic acid and stained with 5% chloracetic acid solution of Coomassie Brilliant Blue in 10% (w/v) trichloroacetic acid.

This technique has been successfully applied to: 1) genetic analysis of electrophoretic variants of perithelial proteins, and 2) to their distribution in single perithecia produced by mycelia heterokaryotic for the determinants of the protein variants and for ascus and ascospore shape. (Supported by Grant GM-12953 from the National Institute of General Medical Sciences, USPHS.) ≠ Section of Botany, Genetic and Development, Cornell University, Ithaca, New York 14853.


We have found that efficient extraction of mycelium, with high yield of intact organelles can be accomplished by a method described here. The procedure described is essentially a modification of the method of Applegate, P.J., R.E. Nelson and R.L. Metzberg.

Mutant enrichment by filtration concentration: a variation for the selection of temperature-conditioned heterocaryons.


The inclusion of sorbose into a medium used for filtration concentration greatly facilitates the separation of growing mycelium from non-growing conidia, and also permits the recovery of individual isolates without the intervening step of plating samples of the primary culture on agar-solidified medium containing sorbose. In addition, because growth is restricted with sorbose, the primary culture need not be held for 12 hours. The following is a general procedure using such a medium for the selection of heterocaryon derivatives with dominant temperature-conditioned alterations.

Conidia from a two-week old minimal medium culture of a stable, nutritionally-balanced heterocaryon are suspended in sterile water, filtered through spun glass wool to remove large mycelial clumps, and exposed to a mutagen (only ultraviolet light has been used). Treated conidia are suspended in 250 ml of Fries minimal salts containing 6% (w/v) sorbose, 0.5% (w/v) glucose and 0.5% fructose in a 500 ml Erlenmeyer flask at a concentration of 1 x 10^6 survivors per milliliter. The culture is agitated at 160 rpm (gyrotary) at a temperature determined to be non-permissive to remove the growing, non-mutant conidia by filtration concentration (37°C for the selection of heat-sensitive derivatives and 35°C for the selection of cold-sensitive derivatives). After 12 hours, the culture is filtered through a combination of gauze-type cheesecloth and spun glass wool such that the porosity of the filter material is decreased at successive filtrations. When no growth is apparent for a period of 24 hours, the culture is shifted to a temperature that will permit the growth of the desired derivatives (239°C for recovery of heat-sensitivies and 279°C C for cold-sensitivies) and agitation continued at 120 rpm. The culture is inoculated at the permissive temperature until conidia that can grow after the temperature shift form 1-2 mm mycelial colonies. The culture fluid is then decanted from the flask and the colonies washed with sterile water and individually cultured under appropriate conditions to confirm their temperature conditional phenotype. Because only minimal medium is used throughout filtration concentration, a high proportion of these isolates are temperature-conditioned heterocaryons (usually between 1/10 and 1/20).

The above procedures have been used to detect mutations in genes whose functions are required for nuclear topoisomerization or for the transfer of information from gene to cytoplasm. ≠ School of Life Sciences, University of Nebraska, Lincoln, and the Department of Physiological Chemistry, University of Wisconsin, Madison.

RESEARCH NOTES

Much of this work has already been reported (Catcheside 1971, Austral. Biochem. Soc. 4:17) and is described here only because of the potential utility of the mutant and the restricted accessibility of the original abstract.

Wild type Neurospora is capable of growing in the presence of high concentrations of structural analogues of number of cellular metabolites. This holds for the genetic dissection of metabolic control processes since the direct selection of analogue resistant mutants may be impracticable. For example, although anthranilate synthetase and chorismate mutase, the allosteric enzymes concerned in the control of chorismate utilization for tryptophan synthesis, are sensitive to 5-methyltryptophan (5MT) in vitro, whole cells are able to grow on media saturated with 5MT (10^-4 M). In order to obtain material from which allosteric mutants affecting the tryptophan sensitive enzymes might be selected, mutants with increased sensitivity to 5MT were sought using filtration enrichment in the presence of 5MT followed by plating ungerminated conidia on medium free of 5MT (Catcheside 1966 Ph.D. Thesis, Univ. of Birmingham, U.K.). Twelve 5MT sensitive mutants were isolated, all map close to y+ linkage group VI and on the basis of recombination frequency are probably allelic 0° one gene: "i."