A pleiotropic mutation in Neurospora conferring sensitivity of analogues of amino acids, purines and pyrimidines

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Abstract
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with the following modifications: using a 50 μ Hamilton syringe, the capillary tube is filled with a separate glycerol solution to a depth of .5 cm, and proportionately twice as much stacking gel is used. Pecier of cellulose acetate film (Sephaphore-I, Gelman), cut to fit into the capillary tube, are washed with 0.1 M phosphate buffer, pH 7.0. A single perithecium is placed 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 Coriolis dielectrophoresis apparatus, at 1/2 mA per gel. After the tracking dye has migrated I cm 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 tubes using a syringe filled with ethylene glycol and provided with a fine needle (No. 27 or 30). The gels are fixed in 10% (w/v) trichloroacetic acid and stained with 0.1% aqueous solution of Coomasie Brilliant Blue in 10% (w/v) trichloroacetic acid.

This technique has been successfully applied to: 1) genetic analysis of electrophoretic variants of perithecial proteins, and 2) to their distribution in single perithecium produced by mycelia heterokaryotic for the determinants of the protein variants and for ascus and ascospore shape. (Supported by Grant GM-12553 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 in less than one minute per sample by using glass powder in a microglass homogenizer of the correct clearance. The clearance of a Dual Tissue Grinder (Kontes Co.) must be enlarged by grinding with silicon carbide abrasive powder until 28 B.S. wire (0.013 inches) can be inserted between the pestle and the wall. To a modified 15 ml Dual Tissue Grinder, 1.0 gram (wet weight) of mycelium and 0.5 gram glass powder are added and homogenized at the bottom of the tube with 5 ml extraction buffer. The grinding pestle is rotated by a low torque motor and constantly raised and lowered during approximately 30 seconds of grinding. Amounts may be scaled up or down by a factor of two with this grinder, and much wider ranges of amounts can be accommodated by using smaller or larger grinders.

The method yields soluble protein concentrations significantly higher than those produced by a more vigorous method previously described (1967. Neurospora News 12:16). However, the present method yields only 50-75% of the total protein per unit of mycelium obtained by the more vigorous method.


The inclusion of sorbose into a medium used for filtration concentration greatly facilitates the separation of growing mycelium from non-growing conidia, and allows 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 only be propagated by the investigator, i.e., 12 hours. The following is a general procedure used in such a medium for the selection of heterocaryon derivatives with dominant temperature-conditional alterations.

Conidia from a two-week-old minimal medium culture of a strain, 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 (w/v) fructose in 500 ml Erlenmeyer flask at a concentration of 10^5 survivors on minimal medium/ml. The culture is agitated at 160 rpm (optimal) 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 150°C for the selection of cold-sensitive derivatives). Every 12 hours, the culture is filtered through a combination of gauzy-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 at which the growth of the desired derivatives (23°C for recovery of heat-sensitive and 27°C for cold-sensitive) and agitation continued at 120 rpm. The culture is incubated 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-conditional heterocaryons (usually between 1/10 and 1/20).

The above procedure has been used to detect mutations in genes whose functions are required for nuclear multiplication 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.

Catcheside, D.E.A. A pleiotropic mutation in Neurospora conferring sensitivity to analogues of amino acids, purines and pyrimidines.

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^-4M). In order to obtain material from which allosteric mutants affecting the tryptophan specific enzyme might be selected, mutants with increased sensitivity to 5MT were sought by 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 more closely related in linkage group VI and on the basis of recombination frequency are probably allelic.

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 many amino acids and of metal cations. This handicap the genetic dissection of metabolic control, but the direct selection of analogue resistant mutants may be impracticable. For example, although the enzymes that catalyze the formation of asparagine from aspartate and glutamate are sensitive to the analogues of asparagine and glutamate, respectively, the high proportion of resistant strains obtained in this way is too low to be useful.

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One allele, mts MN1(s), has been further characterised. The absence of any qualitative change in 5MT catabolism prompted testing for sensitivity to analogues of other metabolites: mts MN1(s) is more sensitive than wild type to analogues of all tested aromatic, neutral and basic amino acids and is also more sensitive to analogues of purines and pyrimidines. Where wild type is inhibited and comparisons can be made, mts MN1(s) is inhibited to a similar degree by between one-tenth and one-hundredth of the analogue concentration effective with wild type. The mutant is not more sensitive to cold, salt or detergent, and the cellular complement of lipids, membrane structural protein and ATP appears normal. The permease systems for 5MT, phenylalanine and arginine are not derepressed, the K₅ for phenylalanine uptake is not grossly affected and efflux is not abolished though significantly longer intracellular pools are maintained following uptake of phenylalanine.

The nature of the change in mts mutants is not clear though alteration to an external or internal permeability barrier seems likely. Like mod-5 (St. Lawrence et al. (1964) Genetics 50: 1383) which overproduce FGSC, the 18:O have been deposited in the FGSC Stock Center: A FGSC #7246, a FGSC #7247. The mutation is conveniently scored in tuber. Strains containing mts fail to grow at 25°C in 72 hours on slopes of Vogel's minimal agar supplemented with 500 μg ml⁻¹ DL-SMT or the appropriate concentration of another amino acid, purine or pyrimidine analogue. Emerson A or a wild type, such as FGSC #691 and 692, is an appropriate mts reference strain. The approximate concentration of analogue in which reducing growth yield of mts in liquid Vogel's medium by 50% in 72 hours is: 5-methyl-DL-tryptophan 4 × 10⁻⁵ M, 8-azaadenine 4 × 10⁻⁵ M, L-ethionine 2 × 10⁻⁵ M, L-carnosine 5 × 10⁻⁴ M, == School of Biological Sciences, Flinders University, Bedford Park, South Australia, 5042, Australia.

Friedman, K.J. and D. Glick. Fatty acid composition of Neurospora plasma membrane.

| TABLE 1 |
| Fatty Acid Composition (%) |
| fatty acid * |
| whole cells | 14:0 | 15:0 | 16:0 |
| plasma membranes | 0.925 ± 0.33 | 0.925 ± 0.15 | 0.305 ± 0.38 |
| fatty acid * |
| whole cells | 16:1 | 16:2 | 18:0 |
| plasma membranes | 5.25 ± 2.1 | 12 ± 2.25 | 1.8 ± 0.75 |
| fatty acid * |
| whole cells | 3.4 ± 0.85 | 6.17 ± 2 | 2.3 ± 0.88 |
| plasma membranes | 18:1 | 18:2 | 18:3 |
| fatty acid * |
| whole cells | 7.6 ± 2.5 | 4.6 ± 2.4 | 4.6 ± 2.6 | 5.3 ± 2.4 |
| plasma membranes | 7.37 ± 3.2 | 4.9 ± 3 | 1.9 | 3.7 ± 2.4 |

*Chain length: unsaturation.

The availability of Neurospora mutants with altered lipid metabolism and the feasibility of using Neurospora with microelectrodes is permitting us to investigate the relationship between membrane lipid composition and membrane electrophysiology (Friedman 1975 J. Membr. Biol. 32: 33-47; 36: 175-190). Such studies assume that the data obtained for total lipid extracts is an accurate reflection of the lipid composition of the plasma membrane. However, we are not aware of any data concerning the distribution of cellular lipids between cytoplasm and cell membrane in Neurospora or other fungi which would support this assumption.

By using the methodology of Scarborough (1975, J. Biol. Chem. 250: 1106-1111) for the isolation of the plasma membrane fraction of the Neurospora cell wall-mutant slime, we have been able to obtain data which demonstrates that the composition of the plasma membrane fraction is in good agreement with the fatty acid composition of total hyphal extracts.

The slime strain was obtained from Dr. Eugene Scarborough and grown on 800 ml of Vogel's minimal media supplemented with 2% (w/v) mannitol, 0.75% (w/v) yeast extract, and 0.75% (w/v) nutrient broth on a rotary shaker (150 rpm) at 31°C. Cells were harvested and washed with buffer four times by centrifugation. Plasma membranes were isolated according to the procedures of Scarborough (1975). Harvested cells and isolated plasma membranes were freeze-dried for 24 hours. Phospholipids were extracted using procedures similar to those detailed by Friedman (1977). Fatty acid methyl esters were obtained using BF₃-Methanol Reagent and identified and quantitated by gas chromato-graphy (see Friedman 1977 for details).

Analyses of three experiments in which fatty acids were extracted from intact slime cells and three experiments in which fatty acids were extracted from isolated plasma membranes are summarized in Table 1 and Figure 1. The means ± standard deviation are shown. To test the hypothesis that there is no difference in fatty acid composition between whole cells and plasma membranes (null hypothesis), we calculated the t-statistic for paired observations. The obtained (0.928) has a probability > 80% (for n = 24). Statistically, therefore, there is no difference between fatty acid composition between whole cells and plasma membranes.

We believe our results indicate that the fatty acid analyses of "whole cell" Neurospora extracts is an accurate reflection of the fatty acid composition of Neurospora plasma membranes.

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