A screening technique for the isolation of macroconidiation mutants

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Abstract
Screening technique for macroconidiation mutants

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cr sn csp-2 and cr sn csp-2\(^2\) derivatives each have useful properties for the examination of individual colonies in a large population. cr sn csp-2\(^2\) colonies can be accurately replica plated with velveteen covered blocks. A single velveteen master is used to faithfully print the location of each colony onto ten or more additional plates by transfer of conidia. There secondary plates can contain media which test directly the properties of the transferred conidio, or which test the properties of colonies that grow from the transferred conidia. Plate cultures of confluent cr sn csp-2\(^2\) colonies are also on excellent source of macroconidia. One 9 cm culture yields ca. 5 \times 10^9 conidia. On the other hand, conidiating colonies of cr sn csp-2 on plates can be exposed individually to any "test" medium, in situ, by adding the medium in a soft agar overlay. The overlay is poured without disturbing the chains of conjoined conidia. Therefore, cross contamination of colonies, via freed conidia, is minimized.

We have capitalized on the described properties of these stocks to isolate single gene mutants which lack NAD(P)glycohydrolase activity (EC 3.2.2.6). (Supported in part by UCLA Medical Sciences Research Fund to P. T. Cohen and on NSF grant to R.W. Siegel.) Department of Biology, University of California, Los Angeles, California 90024.


A rapid and simple method for the detection of cultures defective for the development of wild-type macroconidia is presented. This method provides more efficient detection of mutants than microscopic techniques which are blocked late in the process of conidiation. More importantly, it is one of the few that is easily recognized in the course of routine macroscopic examination; the method described here permits the discrimination between there and wild types.

Cultures are grown in cotton plugged tuber (7 cm \times 1 cm) containing 1 ml Vogel's N + 1.5\% agar for 3-5 days in the light at 35°C. Each tube is then inverted and given a single sharp tap against the metal light shade of a fluorescent lamp. The lamp provider a bright light source so that any conidia mechanically freed are visualized as a cloud of particles falling from the aerial hyphal mass towards the cotton plug.

As an example of the power of the method, a single isolate which produced very few freed conidia was readily detected among ca. 3500 tubs cultures started from mutagenized 74-OR8-1a conidio (see Selitrennikoff 1972 Neurospora News. 19: 23). In agreement, microscopic examination (600X) showed that this culture produces chains of conidio and, relatively rarely, individual conidia. Genetic analysis demonstrated that the phenotype is due to a single gene mutation, csp-1 (conidial separation defective, allele f37) which is tightly linked to arg-3 on IL. Detailed observations of csp-1 and aconidial strains will be reported elsewhere. It may be noted that the method has proved useful for the detection of similar mutants in auxotrophs grown on appropriately supplemented media.

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Smith, B. R. Storage of ascospores in water.

Ascospores may be stored in sterile water for periods of up to a year at room temperature and up to eighteen months at 4°C without appreciable loss in viability. Even longer periods of storage without great loss in viability may be possible, but no tests have been made.

Ascospores are harvested by shaking crosses in sterile water, followed by filtration through two layers of cheese cloth to remove large mycelial fragments. Suspensions are left to stand for 30 min to allow the ascospores to settle out. Excess water, containing conidio, is pipetted off leaving a clean suspension. For storage, 1/2 oz. screw-copped bottle are convenient.

In a series of ten crosses of his-5 X pyr-3 mutants stored for 18 months at 4°C, the loss in viability varied between 2 and 12\% with no detectable change in viability due to genotype. It is not known whether ascospores of other genotypes survive as well when stored for long periods in water.

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Ascospores from desiccated crosses germinate poorly if heat-shocked directly after harvesting or isolation. The low germination can be overcome by rehydrating either in water or on the surface of fresh agar medium. We obtain good results, when isolating from old cross tuber, either by adding sterile water to the tube 12 hours before isolating or by isolating the ascospores from the dehydrated cross tube to fresh slants, which are then left overnight at room temperature (21°C) before subjecting them to heat-shock. (Longer Periods of storage without refrigeration might result in sufficient mycelial growth that heat shocking would not kill all vegetative cells.)

Quantitative data on the effect of dehydration and rehydration were published earlier (Strickland 1960 J. Gen. Microbiol. 22: 585). We are prompted to call attention to the effect once again because it does not seem to be generally known, especially by those beginning to work with Neurospora, and because dehydration can seriously impair efficiency if it is unremedied. In the 1960 report, germination was reduced to less than one-third after 27 days; this was restored to 97.5\% by rehydration.

Rehydration is not necessary when our standard procedure for crosses is followed. Crosses are made on 10 ml slants in large tuber. Spores are ripe and germination is good 27 days after first inoculation. At this time the water loss from evaporation will...