**Selitrennikoff, C. P. and R. E. Nelson.** A screening technique for the isolation of macroconidiation mutants.

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 examination; the method described here permits the discrimination between there and wild types.

Cultures are grown in cotton plugged tuber (7 cm x 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-ORB-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 conidio. 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 aconial strains will be reported elsewhere. It may be noted that the method nor 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 bottler ore 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 dessicated 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...
have shrunk the slant somewhat, but not so as to dessicate the spores. Only if isolation is delayed until the agar has shrunk to half or less its original volume do we take steps to assure that ascospores are hydrated. With crosses in small tubes (10 x 75 mm), drying out occurs quickly and rehydration is more likely to be necessary.

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Westrum, F. M. and N. V. Vigfusson.

A method of NTG mutagenesis of N. crassa.

The following method of N-methyl-N'-nitro-N-nitrosoguanidine mutagenesis has been used to yield on auxotrophic mutant production rate Os high as 17%. This method is particularly useful where other methods of mutagenesis, such as UV light, are unavailable or unadvisable. The wild-type strain STA4, St. Lawrence standard 74A was used for all tests. The NTG is first prepared by dissolving it in distilled water to give a concentration of 3 mg/ml. NTG is slightly insoluble in water and the solution may have to be warmed slightly to aid in dissolving. Freshly prepared solution will give the best results, as NTG deteriorates with age. (At 30 days it is less than 50% as active as fresh). A conidial suspension is then made by washing the conidio from a slant culture using sterile distilled water, filtering it through eight layers of sterile gauze, and adjusting it to a final concentration of 1 x 10⁶ conidio per cc.

Two ml of the NTG solution is added to 100 ml of the 1 x 10⁶ conidial suspension, giving on NTG concentration of 0.06 mg/ml of medium. This is allowed to stand at room temperature for 30 minutes with periodic agitation. A sample is then removed and diluted to a concentration of 1 x 10³/0 conidio/cc. One ml of this concentration is both plated and spread on complete sorbose medium. The plates are then incubated at 25°C for two to three days. When colonies become apparent, replica plating to minimal sorbose may be utilized to rapidly isolate the biochemical mutants. Mutants are then characterized and crossed with a wild-type strain to verify that they are truly mutants.

Table 1. Effect of varying NTG concentration on survival and mutant yield.

<table>
<thead>
<tr>
<th>NTG Concentration</th>
<th>Exposure time (min)</th>
<th>% Survival</th>
<th>% Mutant yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.06 mg/ml medium</td>
<td>5 15 30 45 60 90</td>
<td>45 27 32 22 6 5 35 29 23 19 4 4</td>
<td>17 9 2 8</td>
</tr>
<tr>
<td>0.12 mg/ml medium</td>
<td>5 15 30 45 60 90</td>
<td>45 27 32 22 6 5 35 29 23 19 4 4</td>
<td>17 9 2 8</td>
</tr>
</tbody>
</table>

shorter time. The 0.12 mg/ml concentration, while time-raving, does not reduce the survival percentage as much as might be expected, although it drastically reduces the percent mutant yield. The 30 minute time of mutant yield and the convenience of the shorter time span. Additional tests showed no

The NTG from the conidial suspension with sterile distilled water prior to plating.

Kulaev, I. S. and V. I. Melgunov. Determination of phosphorous in N. crassa extracts.

A method of total phosphorus determination in Neurospora extracts has been described by Hedman (1969 Neurospora News, 14: 10), who suggested the use of a method for total orthophosphate content estimation. However, like all other colorimetric methods of phosphate determination in the aqueous phase, this one seems to be liable to error arising from some disturbing factors (see Berenblum and Chain 1938 Biochem. J.32: 295). Besides which, the time of interaction between molybdate and phosphorous compounds in solution was very long (5-10 min). Thus, the total orthophosphate content will be overstated because of the well-known catalytic effect of molybdate on the hydrolysis of organic phosphates. Therefore, we wish to turn Neurosporologists' attention to another, more advantageous, procedure of phosphate determination.

The method adopted in our laboratory is based mainly on Weil-Malherbe and Green (1951 Biochem. J.49: 286) and Martin and Doty (1949 Analyt. Chem. 21: 965) modifications of the extraction method of phosphate estimation introduced by Berenblum and Chain (1938, ibid. ). The solutions used were: (1) mixture of i-butanol-benzene (1:1, v/v). (2) 5% ammonium molybdate in 4N H₂SO₄, prepared fresh daily by dilution of stock solution of 10% ammonium molybdate in 8N H₂SO₄. (3) Stock solution of stannous chloride; 10 g SnCl₂ dissolved in 25 ml conc. HCl, kept in a brown glass-stoppered bottle at 0°C. (4) Dilute stannous chloride solution; 0.25 ml conc. solution diluted to 10 ml with 1N H₂SO₄ (must be made up fresh when required). (5) Acid ethanol; 10 ml conc. H₂SO₄ + 490 ml absolute ethanol.

Procedure: If the solution to be tested is strongly acid or alkaline, it must first be neutralized to pH 7-8 with NaOH or HCl. Then add 6 ml i-butanol-benzene mixture and 1 ml 5% ammonium molybdate in 4N H₂SO₄ to the test solution made up to 5 ml in a glass-stoppered test tube. Shake it immediately for 15 sec. With a fine-tipped pipette connected to suction flask, discard the aqueous bottom layer as completely as possible. Then add a pinch of anhydrous Na₂SO₄ to the test tube and shake it until the extract is cleared of any emulsified droplets. By means of a syringe pipette, withdraw 2 ml and transfer to a second test tube. Add 2 ml acid ethanol, 0.1 ml dilute SnCl₂ solution and mix by shaking. After 10 min, the intensity of blue color may be deter-