Fungal Genetics Reports

Volume 10 Article 20

Neurospora in the genetics course.

A. M. Lacy

Neurospora in the genetics course.

Abstract

Neurospora in the genetics course.

Lacy, A. M. Neurospora in the genetics course.

While other organisms are used (with some reluctance) in my genetics course, much of the laboratory time is devoted to a long project using Neurospora crassa. The following procedure, which will probably be published in more detail as part of a projected Genetics Laboratory Manual, is designed for use in a course taken primarily by junior biology majors and pre-med students. All of these students have completed the elementary biology course, in which Neurospora is introduced, and most of them have also completed a year of organic chemistry before taking the genetics course.

This type of project is probably feasible only where the number of students enrolled in the lab is less than 30, and where Neurospora research is routinely done in the department. While we have limited our study to tryptophan mutants, this is, in part, a reflection of our own research interests. Presumably mutants involving any well-investigated pathway could be used. About 8 weeks of a 10-week term are necessary for completion of the project. By no means all of the laboratory time is devoted to Neurospora, but 8 weeks' total time is necessary to allow growing time for several repeats of various tests, especially the intra-genic complementation tests.

The general approach involves:

1) Induction (by UV or nitrous acid) and selection (by filtration and selective plating) of tryp mutants.

2) Determination of the particular tryp locus altered in each mutant.

3) Assignment of 3 or 4 tryp mutants (at least 2 of these tryp-3 mutants) to each student. If the yield of new tryp-3 mutants is small, the supply is augmented by "unknowns" from our stock collection.

4) Characterization of the tryp mutants in relation to each other and to known tryp-3mutants on which published data are available. This procedure includes tests for temperature sensitivity, indole and/or indole-glycerol accumulation, ability to grow on indole, linkage to fluffy, and intra-genic complementation.

5) Assay of crude extracts of a wild type and of a tryp-3 mutant for reaction 2 activity of tryptophan synthetase. For this purpose one batch of wild type strain and one of a tryp-3 strain are grown and the lyophilized mycelium distributed to small groups of students working together on the assay.

6) Preparation of a report by each student. This report is written in the form of a scientific paper and includes the characteristics of the mutants studied, how the mutants are related to previously known mutants, possible explanations for conflicting results, etc.

By setting up this project in such a manner that students are studying unknown mutants within a partially structured procedure, it is possible to allow for individuality of evaluation and speculation and for some freedom of experimentation without requiring the additional teaching

staff and the variety of specialized equipment that would be necessary to allow a completely free project.

To present the procedure in detail would be to publish a whole lab manual in the NN. Moreover, the exact procedure differs somewhat from year-to-year, partly in response to availability of equipment, strains, etc., and partly in response to the instructor's need for variety. If any reader desires an amplification of some aspects of this project, I can sometimes be reached by mail, but more surely by telephone. As an example, the procedure by which the students obtain mutants for use in this course is given below.

Neurospora mutant hunt:

When Neurospora (or any other organism) is exposed to a mutagenic agent (such as ultraviolet light, x-rays, nitrous acid, etc.), mutations may occur in many different genes in many different cells. If we wish to keep only the mutated types, especially if we wish to collect only certain of the mutated types, an effective selection technique is required. In this course we will use a local modification of the Woodward and Srb filtration method (1954 Proc. Natl. Acad. Sci. U. S. 40: 192). This method is based on the fact that wild type Neurospora can grow in a simple medium containing only minerals, biotin, and sucrose, while nutritional mutants cannot. After treatment with a mutagen, the conidial suspension is incubated in liquid minimal medium. The wild type conidia will germinate, grow, and can be filtered off; the ungerminated mutant conidia will pass through the filter. After repeated cycles of incubation and filtering, the relative proportion of mutant conidia remaining in the medium will increase. The filtrate is then distributed in petri dishes containing minimal medium supplemented with sorbose (which causes Neurospora to grow in a compact, pellet-like form) and with nutrients required by the desired mutant type. In an ideal experiment, a large proportion of the little colonies which appear on the plates will be of the desired type.

The following procedure will be used for induction and selection of tryptophan-requiring mutants from wild type 74A.

1) Each group of students will be given 5 slants of wild type strain 74A (grown for 5 days on minimal agar medium). Vogel's minimal medium N is used throughout these experiments.

2) Pour 5 ml of sterile distilled water into each of the 5 slants. Disperse the conidia, using a sterile microspatula.

3) Pour suspensions aseptically through a sterile glass wool filter apparatus until about 15 ml of suspension has collected under the filter. A calcium chloride drying tube (the top plugged, the stem wrapped in cotton and inserted in a test tube, and the bulb lined with glass wool) is used for this purpose. Remove the filter and stopper the tube of filtrate with a sterile cotton plug.

4) Pipette, aseptically, one drop of well-shaken suspension onto each side of a haemocytometer. Count the number of conidia in each of 5 "big-squares-containing- 16-little-squares" (demonstration). Average the 5 tallies and calculate the number of conidia/ml of suspension. The

most efficient concentration of conidia will differ somewhat with the mutagen to be used. For UV treatment, 10^7-10^8 conidia/ml is desirable.

5) The induction of mutations:

A. Chemicals. The appropriate concentration of mutagenic chemical is added directly to the conidial suspension and incubated for a given length of time to obtain the desired ratio of mutated to killed nuclei. The action of the chemical is then stopped, usually by the addition of a second chemical which neutralizes its action or by dilution of the mutagenic chemical.

B. Ultra-violet light. Pipette, aseptically, 11- 12 ml of conidial suspension into an empty sterile "deep-dish" petri dish. Quickly replace glass cover. Warm up UV lamp for about 10 minutes. (Do not look at the lamp bulb - the rays can be very damaging to your eyes.) Place the petri dish under the lamp 10 cm below the bulb. (It is desirable to wear rubber gloves while working under the lamp.) The UV source in this experiment is an 8 watt germicidal UV lamp. When you are ready to irradiate your suspension, remove the top of the petri dish (UV rays will not penetrate glass) and, holding the bottom of the dish between thumb and forefinger, rotate gently to obtain maximum exposure of conidia. Time the irradiation and replace the glass petri dish cover (which has been held face down to maintain asepsis). The class will be divided into 5 groups: members of these groups will irradiate their suspensions for 0, 1, 1.25, 1.5, 2 and 3 minutes, respectively.

6) Pipette 5 ml of treated suspension into each of 2 sterile 1-liter Erlenmeyer flasks with filter tops containing 250 ml of minimal medium. Add 0.25 g of streptomycin sulfate " as aseptically as possible". The filter-top plugs are made by placing a rectangular strip of curity 60 cheesecloth across the mouth of an Erlenmeyer flask, then placing the same sized strip across the mouth at a right angle to the first, and then plugging the flask with cotton so that the cheesecloth is pushed down into the flask but the ends are left above it. This makes it possible to remove the plug easily and yet be left with a layer of cheesecloth for filtering.

7) Label flasks with all pertinent information and incubate flask X at 25C and flask Y at 30C.

8) Now for the filtration part of the procedure. The most efficient way of handling the flasks for rapid and aseptic transfer from a flask of liquid, through a sterile cheesecloth filter, and into a sterile empty flask will be demonstrated. The 0 time suspension should be filtered at approximately 9 hours after inoculation. Both the 0 time suspension and the treated suspensions should be filtered at approximately 8 AM, 2 PM and 9 PM for the next two days. Probably one filtration each is sufficient for the 3rd and 4th days. Note: 24 hours after inoculation, filter into flasks containing 100 ml of sterile minimal medium. All other filtrations should be into empty flasks.

9) The time of filtrate plating (usually after 5 days of filtration or when 12 hours passes with no formation of new mycelia) will be announced in class. Small aliquots of filtrate (the exact amount depending on the concentration of treated suspension and the length of time treated) will be pipetted, aseptically, into flasks of molten, but fairly cool (approximately 45C) minimal sorbose agar (1% sorbose, 0. 1% sucrose) containing (since we are selecting

for tryptophan requiring mutants) about 25ug L-tryptophan/ml. The agar suspension will be distributed over 10 already-layered plates for each X and each Y flask, and the plates will be incubated at 25C and 30C, respectively.

10) The plates should be inspected at least once daily (preferably twice) and any visible spots of growth cut out of the agar (aseptically) with a microspatula, inoculated into small tubes of tryptophan-supplemented agar, and incubated at 30C until good growth is obtained (about 3 days). Draw a crayon circle on the petri dish bottom around the spot picked so that you and your lob partners do not repick the some colony later.

11) Transfer, aseptically, tiny wisps of conidia from each agar slant to appropriately labelled small tubes of minimal liquid medium; make two tubes per presumptive mutant. Incubate one at 25C and the other at 37C. Save presumptive mutant stocks in the refrigerator.

12) Those isolates which DO NOT show significant growth on minimal medium at bothtemperatures after 2 days' incubation can be assumed to be mutated in some gene controlling tryptophan formation. Why? Check with the instructor as to what constitutes "significant growth". The mutant stocks should be numbered, transferred onto 3 large tryptophan-supplemented slants, and incubated at 30C.

13) At this point, the tryptophan mutants obtained by the class will be supplemented by tryptophan mutants obtained by the instructor, so that each student will have 3 unknown tryptophan mutants. Study and characterization of these mutant strains will constitute the central experiment of the term's laboratory work.

- - - Department of Biological Sciences, Goucher College, Towson, Maryland 21204.