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Neurospora in the freshman biology course.

Abstract
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Lacy, A. M. Neurospora in the freshman biology course.

The following laboratory procedure and description of necessary materials is taken from a new "Labtext" From one cell to many, by Funk and Lacy 1966 Wm. C. Brown Co., Dubuque, with permission of authors and publisher. Some introductory material has been deleted.

This manual is designed for use in a one-semester elementary biology course devoted to the "cell to organism" approach. Cell structure and metabolism, genetics, plant and animal development, and , finally, the structure of a multicellular animal (the dogfish shark) are dealt with in this course.

The students begin the Neurospora experiment during the second half of the course, after they have each completed a four-week independent project using a microorganism. Consequently, they are reasonably proficient in aseptic technique and use of the microscope. This experiment does not depend for its success on extensive first-hand knowledge of Neurospora on the part of the instructor. Moreover, this experiment, in fact this whole manual, is primarily designed for colleges which wish to present a modern course in biology but which lack both a large staff of graduate teaching assistants and an unlimited supply of specialized equipment. The experiment occupies about one hour of one laboratory period (of which the other two hours are devoted to corn genetics, blood typing and blood cell identification), about 15 minutes of a second laboratory period and about five minutes of a third period.

Materials:

(per student) Dissecting microscope with 45-60X magnification

1 glass-handled dissecting needle

1 metal-handled microspatula

1 small flask of 95% alcohol

1 microscope slide

10 (10 x 75 mm) tubes of supplemented agar medium (medium N)

10 (10 x 75 mm) tubes of minimal liquid medium (medium N)

(per 8 students) 2 tubes of a Neurospora cross (biochemical mutant x "morphological mutant")

1 petri dish of 4% water agar

1 wire test tube rack for small (10 or 12 x 75 mm) test tubes

(per class) 1 large water bath, set at 60C

Procedure:

Anatomy of Neurospora: Each group of four students will be given a test tube in which two mutant strains of N. crassa have been crossed. Observe the different structures in the tube with the dissecting microscope. Also, remove some of the organism aseptically, place it on a slide in wet mount, and observe under the compound microscope. Identify the vegetative structures (mycelium, hyphae, asexual conidia) and sexual reproductive structures (perithecia, ascospores).

Analyzing progeny from a Neurospora cross: By isolating random ascospores (haploids) from the cross, allowing them to germinate and grow, and then studying the characteristics of each isolate, it should be possible to determine whether or not the two genes involved are linked together on the same chromosome and, if so, how closely linked they are. For this experiment, each student will isolate and study 10 ascospores. When the ascospores are block and ripe, they are shot out of the perithecia and form small black clumps on the upper side of the test tube. Dip the microspatula into the alcohol, flame, and use it to remove a few spores from the side of the tube. Place them on one end of a 2 cm x 3 cm agar block on a glass slide. Note: agar dries out very rapidly; do not leave this cut piece lying around too long before using. Remember to practice aseptic transfer techniques when dipping into the test tube containing the cross. Aseptic technique is, of course, not possible when working on the agar block.

After flaming your microspatula, cut one side of the agar block into 10 small squares as demonstrated by the instructor. Then, using the glass-handled dissecting needle (**DO NOTFLAME**), and using the highest power magnification of your dissecting microscope (45-60x), place one black spore on each square of agar. With your flat microspatula, pick up each little agar square individually and place right side up in individual small test tubes of supplemented agar medium. Label each tube with your name, the cross used, and an isolation number.

These small test tubes containing the isolated spores should be placed in a 60C water bath for 40 minutes. This treatment kills contaminants which may have fallen onto the agar during the isolation and all parental mycelia and conidia that may have stuck to the ascospores. It also provides the "heat-shock" necessary for initiation of spore germination. After "heatshocking", the tubes should be incubated at room temperature until the next laboratory period (or for at least 4 days).

During the next laboratory period, the following procedures should be carried out: 1) The germination percentage should be determined. 2) The tubes containing germinated spores should be examined and the morphology of the organism in each tube recorded. The instructor will demonstrate to you the different types of morphology you may expect to observe. If one parent in the cross was an albino mutant, set the tubes in the light for at least one hour before classifying. Carotenoid synthesis in Neurospora is light dependent. 3) A few conidia or small bits of the mycelium from each tube should be aseptically transferred to tubes of minimal liquid medium (a medium which will support the growth of wild type Neurospora, but not of biochemical mutants).

These inoculated tubes of minimal medium should be incubated at room temperature for two or three days. Then the following points should be determined. 1) Which tubes showed growth and which did not? 2) What does this tell you about the phenotypes of these progeny? Genotypes? 3) What is the correlation between the morphological characteristics of the progeny and the biochemical characteristics (as determined by growth tests on minimal medium)? 4) Is the gene locus determining the morphological character linked to the one determining the ability to synthesize a certain nutrient factor? At the end of the laboratory period, pool your data with that of others in the class studying the same cross, so that you can determine with some statistical accuracy whether or not the two genes are linked.

Before the ascospores are shot out of perithecia, they are contained in sacs called asci (eight spores to each) and are arranged in order of meiotic segregation within these asci . Why do people often isolate spores in order from the asci? If you wish, you may try to do this yourself. Remove a ripe, black perithecium from the crossing tube, squeeze it open with forceps, pull an ascus with eight ripe, b1ack spores to a clean part of the agar, and dissect in order with a microneedle. This requires a steady hand.

References:

Barish 1965 The gene concept, p. 82-86. Reinhold, New York; Beadle 1948 Sci. Am. 179:30; Bonner and Mills 1964 Heredity, p. 18-28. 2nd ed. Prentice-Hall, New York; Sinnott, Dunn and Dobzhansky 1958 Principles of genetics, p. 173-174, 321-322, 333-336. 5th ed. McGraw-Hill, New York; Srb, Owen and Edgar 1965 General genetics, p. 98-102. 2nd ed. Freeman, San Francisco.

Preparation of materials:

The glass-handled dissecting needles are made by inserting 00 Genuine Bohemian Insect Pins (Carolina Biological Supply Co. #A740) into the melted ends of hollow soft glass rods (diam. 5 mm; length about 6 inches) and then flattening the glass onto the pin with forceps. The needles are most easily stored by passing them through a rubber stopper and plugging the stopper into an 18 x 75 mm test tube.

The metal-handled microspatula is made by hammering a 3.5 inch piece of nichrome or chromel wire (24 gauge) until the tip is more or less square, cutting the edges off sharply, and inserting the wire into any fairly short inoculating loop holder.

Test tube racks can be made by buying wire fencing of the appropriate mesh, cutting into two rectangles and a square, and weaving or welding the broken ends together to make a three-tiered rack.

Minimal media for Neurospora are available from Difco (0324-15, 0460-15, 0817-01). We have not tried these, but they would probably be convenient for course work. The most commonly used vegetative medium for Neurospora is medium N or Vogel's medium (Vogel 1956 Microbial Genet. Bull. 13: 42). This is generally made up 50x and diluted as needed.

The supplements added to the medium will depend upon the mutants used. 150 ug/ml of L-amino acid is usually sufficient for growth of any amino acid mutant, but for crossing and germination higher concentrations in the range of 200-300 ug/ml are often more satisfactory.

Neurospora strains of all kinds are available free of charge (in limited numbers) from the Fungal Genetics Stock Center, Dartmouth College, Hanover, New Hampshire. Albino is one of the most satisfactory morphological mutants for class use. Almost any amino acid mutant is suitable for the biochemical mutant. We usually use albino and a tryp-3 mutant as parents. Be sure to order parents of different mating types (A and a).

Neurospora requires a special medium for crossing. A synthetic crossing medium(Westergaard and Mitchell 1947 Am. J. Botany 34: 573) is generally used in research, but corn meal agar with dextrose (Difco B114) is probably simpler to use for course work. The best procedure is to inoculate one parent onto a slant of corn meal agar and incubate at 25C for five days. At that time, dust the conidia from the second parent over the mycelium of the first and reincubate at 25C. While two weeks should be sufficient to obtain ripe spores, it is wise to start crosses a month or two before needed. When ripe black ascospores appear in clumps on the upper inside surface of the tube, the cross may be stored in the refrigerator until needed. Refrigerated crosses will retain high viability for as long as a year.

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