Fungal Genetics Reports

Volume 10 Article 15

Growth of Neurospora

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Abstract

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The following exercise is taken from "Principles of genetics Laboratory manual" by Hartman, Suskind and Wright (Wm. C. Brown Co., 1965) and is presented here with the permission of the authors and publisher.

The growth of fungi.

The purpose of this experiment is twofold. First is to examine the morphology of an ascomycete, and second, to become acquainted with the concept and use of nutritional mutants. As you know, biochemical genetic studies have relied heavily on the use of the bread mold,Neurospora crassa. The widespread use of Neurospora in biochemical genetic work is based on its rapid growth and development during versatile life cycles, its well characterized genetics (particularly at meiosis), and its simple nutritional requirements. It can be readily propagated asexually, and large populations of a particular genotype can be easily obtained.

Neurospora (wild type) will grow on a medium containing inorganic salts, a carbon source such as sucrose, and one of the B vitamins, biotin. These substances constitute the "minimal" medium, and from these components, Neurospora can synthesize all of its protoplasmic constituents including vitamins, amino acids, purine, pyrimidine and fats.

It is known that mutation can often be recognized phenotypically by the loss of the capacity of the organism to carry out a particular biochemical reaction. This is frequently accompanied by the appearance of a specific nutritional requirement reflecting the effect of the "genetic block". For example, a mutant strain may require a particular vitamin, amino acid, etc. Such mutants are termed "auxotrophic" strains. In the absence of the specific growth factor required by the mutant, the mutation becomes lethal, i.e., the organism cannot grow.

In today's experiment you will set up a bioassay for vitamin B_6 (pyridoxine) using a pyridoxineless mutant, Y-2329, of Neurospora. Three points will be emphasized in this experiment. (Each pair of students will set up the experiment.)

1. The nutritional independence of a wild type of strain, 5297 (i.e., growth on minimal medium without pyridoxine).

2. The nutritional dependence of mutant Y-2329 (i.e., no growth on minimal medium without pyridoxine).

3. The quantitative growth response to varying concentrations of pyridoxine (i.e., microbiological assay for pyridoxine).

A. Each group of students will be provided with a conidiated slant of wild type strains 5297a and the pyridoxine mutant V-2329. For each slant, transfer a loop-full of conidia to a tube containing 5 ml of sterile distilled water and vigorously mix until a suspension of conidia is obtained. These will be your inocula for the growth experiment.

B. 1. 5297a experiment.

Add 20 ml of minimal medium to each of three flasks (125 ml Erlenmeyer), stopped with cotton plugs and autoclave.

2. Y-2329 experiment.

a. Add 20 ml of minimal medium to each of three flasks (125 ml Erlenmeyer), stopped with cotton plugs and autoclave.

(Minimal control)

b. Add 20 ml of minimal medium to each of 10 flasks (125 ml Erlenmeyer), using a pyridoxine stock solution (l ug/ml), add pyridoxine concentrations ranging from 0.1-1.0 ug pyridoxine/flask. Stopper with cotton plugs and autoclave.

C. Place flasks in cold room for 15 minutes. After the flasks have cooled to room temperature, inoculate flasks from B-1 with 2 drops each of the wild strains 5297a conidial suspension. Gently swirl flask contents. The flasks from B-2a, b are similarly inoculated with the conidial suspension of the pyridoxine mutant, Y-2329. Use sterile 1.0 ml pipettes for the inoculation of the flasks.

D. Incubate the flasks at 30C for 72-96 hours. The flasks can be stored in the cold room until next lab.

E. Harvest and weigh the mycelia. The mycelia are fished out of flasks using a small spatula. Sterile technique is not necessary at this point. Squeeze the pads as dry as you can and place each pad in a numbered depression of a spot plate. Dry in the oven (100C) for 3 hours. Weigh the dried samples on an analytical balance.

F. Plot your data, i.e., a pyridoxine response curve: mg. dry weight per pad vs. pyridoxine concentration.

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Materials per 2 students:

96-120 hr. slant of 5297a - 1

120 hr. slant of Y-2329 (B_6) - 1

test tube with 5 ml sterile distilled water - 2

1.0 ml pipettes - 3

125 ml Erlenmeyer flasks - 16

baskets and trays for carrying flasks

pyridoxine stock solution 1 ug/ml - 5 ml

spatula

porcelain or glass spot plate - 2

non-absorbent cotton

minimal medium (incl. 2% sucrose) - 350 ml

References: Straus 1951 Arch. Biochem. 30:2912; Wagner and Mitchell 1964 Genetics and metabolism. 2nd ed. Wiley; p. 163. Vogel and Bonner 1958 p. 1 In Ruhland (ed.) Encyclopedia of plant physiology. Springer; Fincham and Day 1963 Fungal genetics. Davis.

Medium: Fries minimal medium.

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