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Abstract

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Using these procedures, DNA of high molecular weight and good purity can be obtained. If desired, the identification and quantitation of the isolated substance can be made by means of UV-spectrophotometry or chemical tests, such as the diphenylamine reaction. For a 2-hour laboratory period, the procedure can be carried through step 8. The product at this stage is DNA with adhering protein and RNA. The experiment may be terminated at this point or else the DNA can be dissolved in 0.1 SCC and stored in the refrigerator and the isolation can be continued during the next laboratory period.

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The laboratory exercise given below has been used successfully for a number of years in a genetics course taken primarily by graduate students, but could readily be used in an undergraduate course, if desired.

Preliminary statement:

Some Neurospora mutants accumulate metabolic intermediates in the mycelium or in the surrounding medium. The indentification of such intermediates provides important clues concerning the nature and the genetic control of certain biosynthetic pathways. The object of this exercise is the demonstration of the accumulation of anthranilic acid by a tryptophanless mutant of Neurospora. Anthranilic acid is an intermediate in the biosynthesis of tryptophan (Wagner and Mitchell 1955 Genetics and metabolism, p. 310. 2nd ed. Wiley, New York).

Strains used:

Strain 10575. This strain is defective at the tryp-1 locus. It grows if supplied indole or tryptophan, but is not able to utilize anthranilic acid for growth.

Strain B1312. Judging by its growth responses, this strain appears to be mutant at the tryp-2 locus. Other tryp-2 mutants (e.g. 75001 or 40008) would probably be equally useful in this experiment. Mutants of the tryp-2 type are able to utilize anthranilic acid, indole, tryptophan, or certain other related compounds for growth.

Both mutant strains are readily cultured on slants of Fries' minimal agar supplemented with L-tryptophan at a concentration of 0.1 mg/ml. Cultures approximately 1 week old should be available for the required inoculations.

Equipment and supplies:

Fries minimal medium. (Other minimal media commonly used for Neurospora would probably be equally satisfactory).

Anthranilic acid and L-tryptophan. At a concentration of 1 mg/ml these compounds dissolve quite readily in water. Warming speeds solution. Dilute anthranilic acid to 0.2 mg/ml for convenience in bioassay.

Sterile water. Add 1 or 2 ml of water to each of several 4-inch test tubes, plug with cotton, and autoclave.

Pasteur pipettes. These are conveniently made by cutting 7-mm soft glass tubing into 15 cm lengths, plugging both ends of each length with cotton, and autoclaving. Shortly before use, heat the center of each length to softness in a Bunsen flame and pull out to form 2 pipettes.

Erlenmeyer flasks, 125-ml. Nine flasks will be needed for each student or pair of students.

Pipettes, 1-ml, graduated. Each student, or pair, needs 2.

Graduated cylinders, 25-ml . Each student, or pair, needs 1 .

Chromatography paper, 6 x 11-inch sheets of Whatman No. 1. Several students may use the same sheet of paper.

Chromatography vessels. One is needed for each sheet. Gallon jars may be used, but standard 6" x 12" chromatography jars are more convenient. Saranwrap is satisfactory for covering the jars.

Solvents for chromatography, about 100 ml per vessel. Two freshly prepared solvents are needed:

n-propyl alcohol - 1% ammonia (2:1, v/v) and

n-propyl alcohol - 1% acetic acid (2:1, v/v)

Glass capillaries. These may be drawn out from 7-mm soft glass tubing.

Ultraviolet lamp or "Blacklight". Peak emission of the lamp should be near 360mu. Autoclave.

Drying oven, 80C.

Analytical balance.

Procedure:

Prepare, and plug with cotton, the following flasks; a) 20 ml minimal medium, b) 20 ml minimal + 0.25 mg anthranilic acid, and c) 20 ml minimal + 0.25 mg L-tryptophan. Autoclave the flasks 20 min. at 15 psi, cool, and inoculate each flask with 1 drop of a suspension made by dispersing a small amount of 10575 conidia in sterile water. Incubate the flasks at room temperature or in a 25C incubator if one is available. The response of strain 10575 to minimal medium, to anthranilic acid, and to tryptophan is shown by the growth or lack of growth occurring in the 3 flasks. The medium in flask c, after growth of strain 10575, will be used in next week's laboratory.

After an incubation period of 7 days (4 to 7 days should be satisfactory, depending on the laboratory schedule), observe the 3 flasks under the ultraviolet lamp. The blue fluorescence of the medium in flask c is produced by a number of substances, perhaps the chief of which is anthranilic acid. Proof of the identity of this compound would require its isolation in pure form from the medium. Such isolation will not be attempted as a part of this exercise, but a partial characterization and assay of the compound will be made on the basis of paper chromatography and biological activity.

A. Paper chromatography. Using a glass capillary, apply a spot of the medium from flask c to a previously marked position on a line 3/4 inch from one of the long edges of a 6 x I 1-inch sheet of Whatman No. 1 filter paper. Sufficient medium should be applied to make the spot approximately 1/4 inch in diameter. Let the spot dry, then repeat the application. Continue this sequence of spotting and drying until 10 applications have been made at the same position. As a control, make one application of the anthranilic acid solution having a concentration of 1 mg/ml . The control spot should be applied along the base line about 1 inch from where the medium was applied. Control spots of additional fluorescent compounds may be used if desired. (Also, the chromatographic separation of the constituents of washable black Skrip ink makes a rather striking demonstration which can be observed while the solvent is traveling up the paper.) After all spots are dry, staple together the ends of the sheet to form a cylinder 6 inches high. Do not permit the ends to overlap each other. Place the cylinder in one of the solvent vessels provided, and cover the vessel. Part of the class will use the n-propyl alcohol-ammonia solvent and the remainder will use the n-propyl alcohol-acetic acid solvent. After approximately 2 ½ hours, remove the chromatograms from the solvents, dry, and examine them under the ultraviolet lamp. Mark the solvent front and any fluorescent spots that are apparent. Measure distances from the base line to the solvent front and to the centers of any fluorescent spots. Calculate the Rf value of each spot as the ratio of the distance traveled by the solute to the distance traveled by the solvent. Each student should observe and record results obtained with both solvents.

B. Bioassay. Prepare and autoclave the following flasks: 1) 20 ml minimal medium, 2) same, + 1 ml medium from flask c, 3) same, + 0.02 mg anthranilic acid, 4) same, + 0.05 mg anthranilic acid, 5) same, + 0. 10 mg anthranilic acid, and 6) same, + 0.20 mg anthranilic acid. Inoculate each of the 6 flasks with 1 drop of a conidial suspension of strain B1312 (or other tryp-2 mutant). Incubate at room temperature (or 25C) for 4 days, then harvest the mycelial pads, squeeze out most of the liquid, dry the pads overnight at 80C, and weigh them to the nearest mg. Plot dry weights against quantity of anthranilic acid, for flasks 3, 4, 5, and 6. Assuming that anthranilic acid is the only material in 10575 culture filtrates with activity for strain B 1312, calculate the