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Abstract

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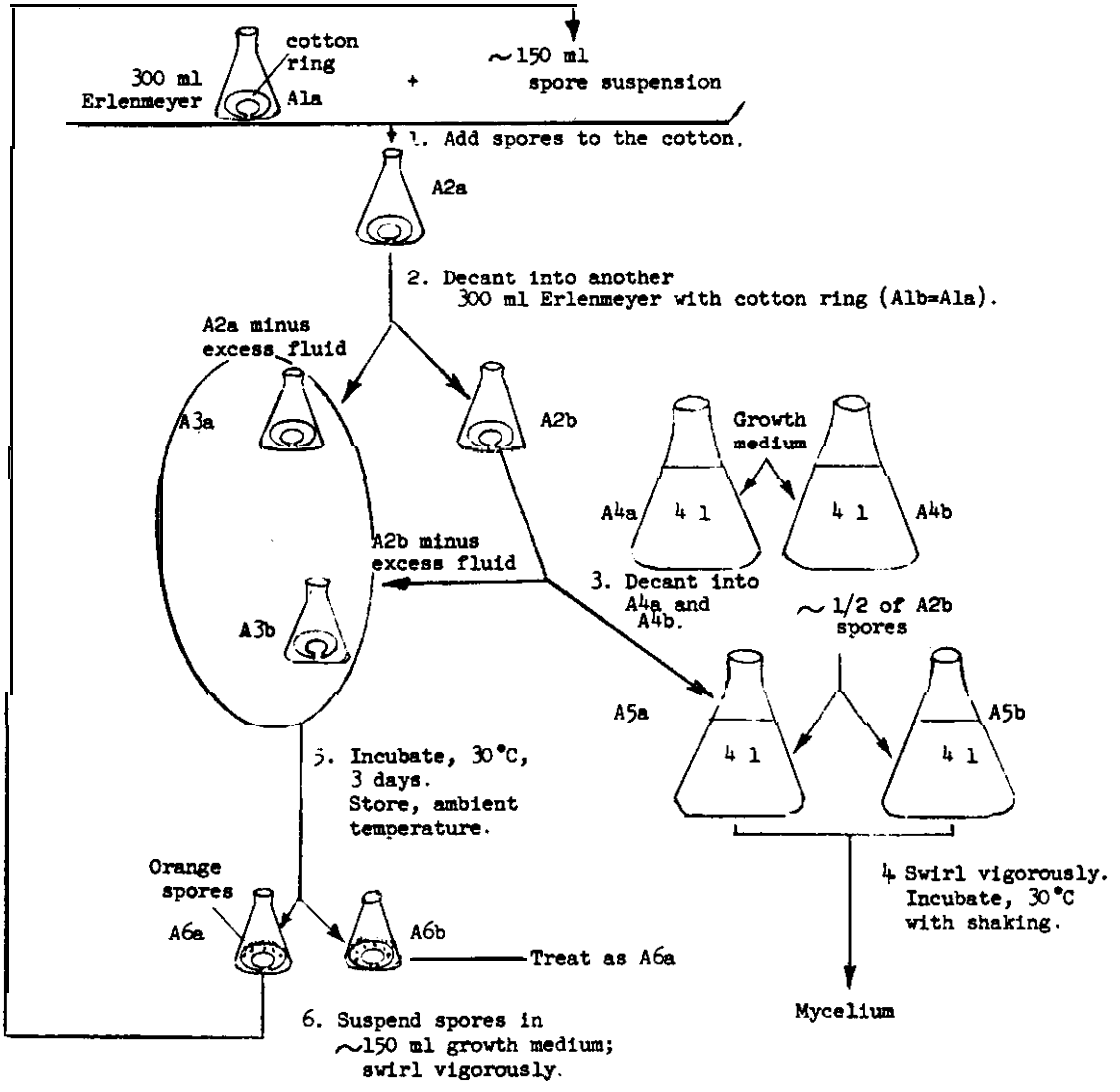
Boker, N. Growth of N. crassa conidia and mycelium on a single medium.

N. crassa conidia ore frequently cultured on agar slants and then transferred into a liquid medium for the growth of mycelium. In order to obtain large quantities of mycelium, it is customary to go through 2 transfers (small to large volume) since the quantities of conidia obtainable from short-term slant cultures is usually too small to initiate mpid growth of mycelium in a large volume of liquid medium. I hove observed that conidio of N. crassa, when suspended in Horowitz and Beadle's modified Fries medium (1943 J. Biol. Chem. 150: 325), are odrorbed by wet or dry cotton and that large numbers of conidia ore produced on the cotton. These observations led to the development of a simple and rapid method for producing large quantities of both conidia and mycelium, using a single growth medium. The method eliminates the need for agar slant cultures as well as for intermediate transfers through a liquid medium. Morover, the procedure con be cyclic, for each time that conidia ore suspended in liquid medium to initiate mycelial growth, the some spore suspension con also be induced to form large quantities of new conidio on cotton.

The cyclic procedure is diogrommed in Figure 1. N. crassa (strain 74a) conidia grown on agar slants were suspended in

Figure 1. Method for cyclic growth of N. crassa conidio and mycelium on a single medium.



approximately 150 ml of modified Fries medium and the suspension was used to initiate the growth cycle. Thereafter, it was no longer necessary to produce conidia on agar slants. Flasks Ala and Alb were prepared as follows: A cotton rectangle was cut from a sheet and rolled into a cylinder having a length which equaled the height of the Erlenmeyer flask. Before placing the cotton into the flasks, each cotton cylinder was washed with distilled water, and the excess moisture was squeezed out. At this point the diameter of the wet cotton cylinder was just slightly less than that of the Erlenmayer flask's mouth. The damp cotton cylinder was then forced to the bottom of the flask with a heavy gloss rod so that it formed a tightly wedged ring (Fig. 1, Flask Ala). The cotton was washed several times by adding and decanting distilled water; during this procedure the cotton remained wedged in place. Care was taken at steps 2 and 3 (after inoculation with a conidial suspension) to decant as much of the medium as possible to minimize mycelial growth in A3a,b. Note that step 2 (the seeding with conidio of a duplicate, cotton-containing Erlenmeyer flask (flask Alb)) is optional and may be omitted. After several doys at 30°C and several doys storage at room temperature, the upper surface of cotton in flasks A3a,b become covered with a thick layer of orange conidia. Growth of conidia in each "cotton-flask" appeared to be highly reproducible. Most of the conidia were readily dislodged from the cotton at step 6 by vigorous swirling; however, the resulting spore suspension was inhomogeneous and contained many large clumps. A dark crust remained an the cotton and this interfered markedly with the further development of conidio in the original cotton-flasks, thereby necessitating transfer over fresh cotton in order to continue the conidial culture.

About half of each spore suspension was usually decanted into 4 liters of sterile growth medium in each of two 5-liter Erlenmeyers (step 3). The suspension was then swirled vigorously in order to obtain a more homogeneous suspension. The mycelial cultures were then grown at 30°C in air on a rotary shaker. The growth rote of mycelium in the 5-l flasks (A5a,b) was also highly reliable, even though the spore concentration in the inoculum was never measured and standardized. Apparently the spores are in excess in every core and some other factor, such as oxygen availability, limits the growth rate under these conditions.

Two aspects of the present method which make it especially useful ore the time roved and the reproducibility of growth of both conidio and mycelium. Only several minutes are required to inoculate several new conidial cultures and, at the same time, 8 liters of fluid medium for mycelium production. Moreover, the mycelium war obtained regularly in a highly dispersed form (like white caterpillars) without one's having to shake the flasks vigorously at frequent intervals during the early stager of growth. This contrasted sharply with our experiences using Fernbach flasks, in which the agitation of a rotary shaker was insufficient to prevent clumping of the mycelium. Although conditions were not explored to optimize the yield of conidia, it appears that, given a large enough container to expose a vast surface of cotton, one should be able to produce within several days massive quantities of conidio for experimental purposes.

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