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Abstract

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We have utilized the following procedure for obtaining yields of young Neurospora hyphae of several hundred grams wet weight in 12 hours growing time. Conidia from the Chilton a strain of N. crassa are produced in 2600-ml Fernbach flasks on layers of agar containing the medium: 0.5% yeast extract, 1% sucrose, and salts at the concentration of Fries" #3 solution (Ryan. Am. J. Bot. 30: 784, 1943). To insure free circulation of air, the flasks are covered with layers of cotton between gauze held in place by rubber bands. After one week, the agar surface is washed with sterile water; the conidial suspension is filtered through glass wool to remove mycelial fragments and its optical density is measured. The number of spores is determined by referring to a standard curve relating optical density to condia concentration. A volume of suspension containing 1.8×10^{10} spores (approximately 2/3) the yield from one Fernbach flask) is inoculated into a 6 liter flask containing 3-1/2 liters of the complex medium to which a silicone antifoaming agent has been added. The flasks are vigorously aerated at room temperature. To prevent formation of mats of hyphae along the walls, each flask is stirred by a magnetic stirrer (but thermally insulated from it by a sheet of rock wool).

Under these conditions, germination begins after 2 hours, and the total number of germinated conidia increases at a nearly linear rate until a maximum of 90% germination is reached at 8 hours (curve A, Figure 1). An even more rapid germination of conidia may be obtained if the cultures are first kept oxygen-free for 5 hours and then aerated. In these cultures, germination appears to begin immediately upon aeration and reaches a maximum 6 hours later (curve B, Figure 1). Since the total time involved in the latter case is longer, we usually aerate our cultures immediately and harvest the hyphae after 12 hours. At this time, the hyphae are still largely unbranched and the small clumps that occasionally occur result from the intertwining of several independent hyphae rather than from the branching of single hyphae.

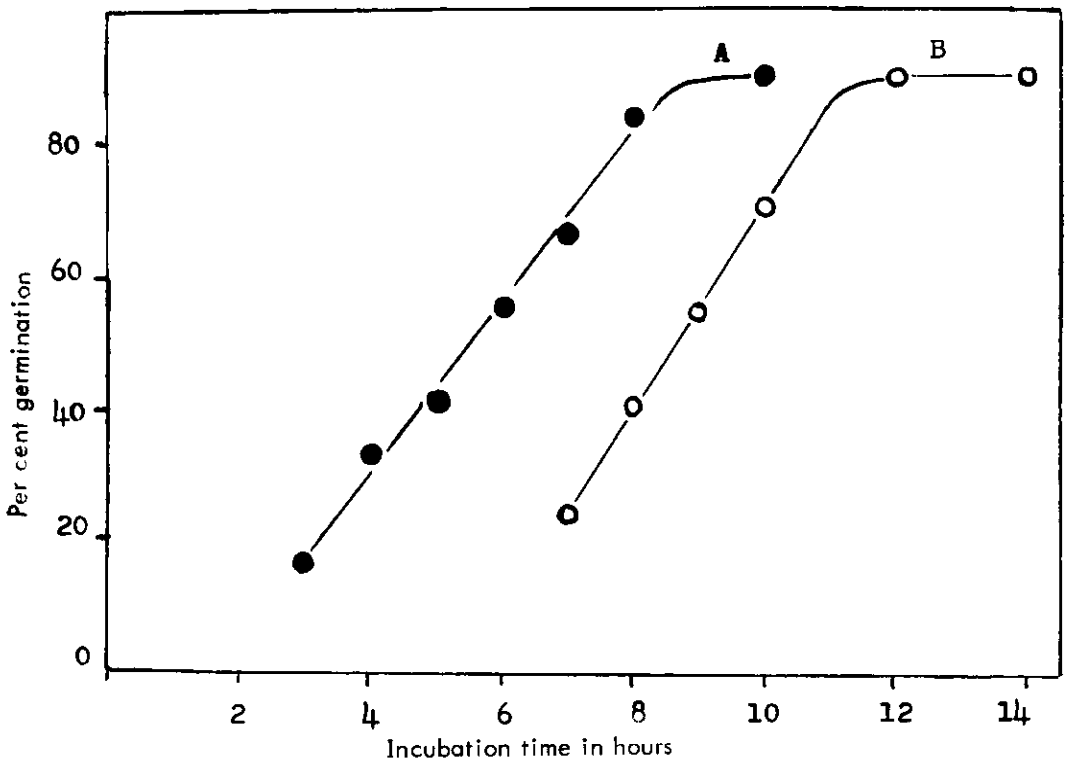

Figure 1. Time course of conidia germination. Curve A: aerated at time of inoculation. Curve B: flushed with N_2 and kept oxygen-free until 5 hours after inoculation and then aerated. Each percentage is based on counts of more than 500 spores.

Hyphal extension in a 12 hour culture showed a median length of 96 microns and a range of 56 to 144 microns encompassed between the first and third quartiles (Figure 2).

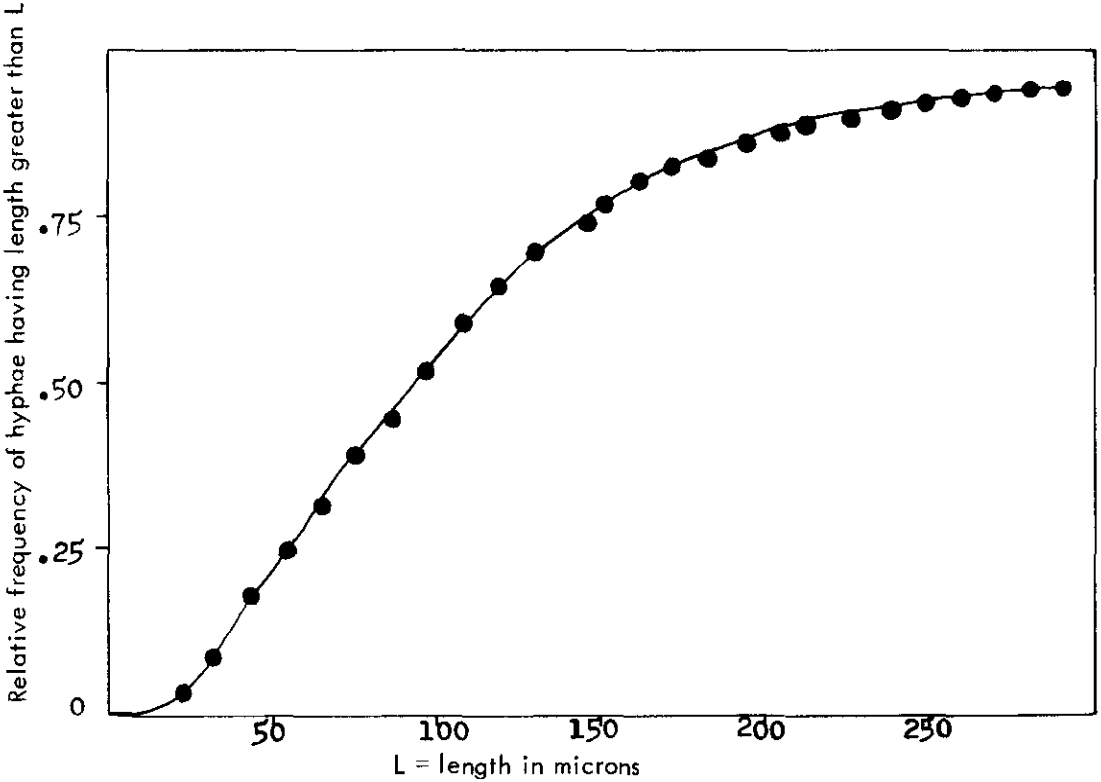

Figure 2. Cumulative distribution of hyphal lengths from a 12 hour culture. Based on measurements on 400 individuals. The values of the first quartile, median, and the third quartile are 56, 96, and 144 microns, respectively.

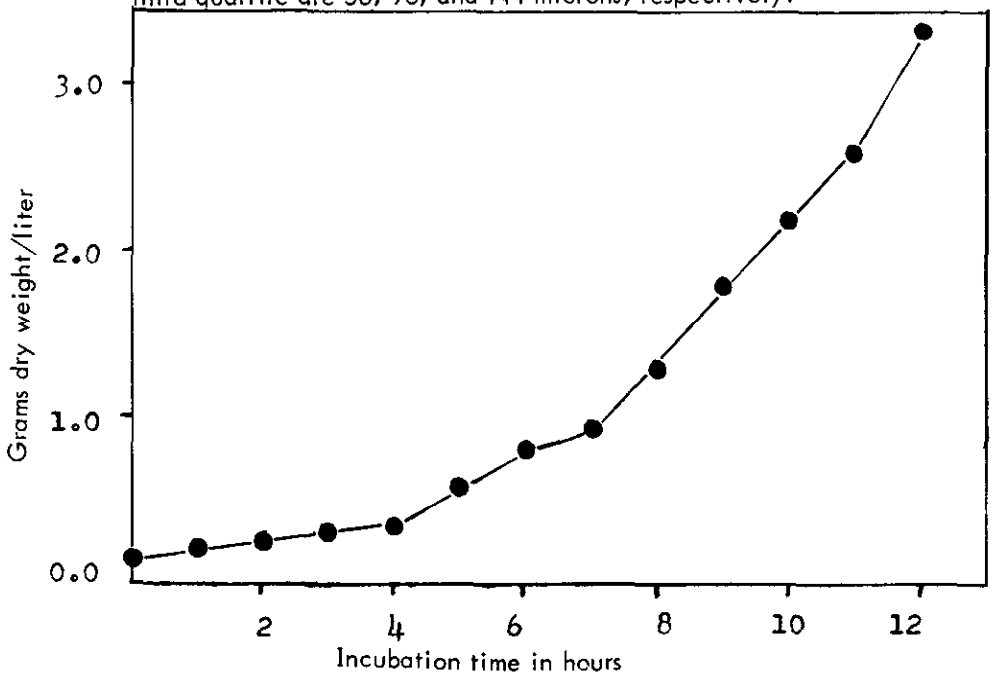

Figure 3. Growth curve at 25 degrees centigrade.

Figure 3 shows a growth curve for a typical culture. The dry weight increases at first slowly, and then more rapidly, as the conidia germinate. As maximum germination is approached, the growth rate becomes linear and remains so until 11 to 12 hours, when branching begins to occur. This culture would produce 100 grams of hyphae, wet weight, at 12 hours from 3-1/2 liters of medium. (U. S. Public Health Service Traineesunder grant 2G-714). --Adolphus Busch III Laboratory of Molecular Biology, Washington University, St. Louis, Mo.