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Observations of perpetual hyphal propagation in Neurospora crassa

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Abstract
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Certain regularly observed degenerative changes in aged cultures of Aspergillus appear to be of cytoplasmic origin (J. L. Jinks, Compt. rend. Lab. Carlsberg, Ser. physiol. 26: 183-203, 1956); one may question whether similar changes occur in Neurospora cultures. Hoping both to answer this question and that such Neurospora cultures might provide a source of spontaneous cytoplasmic mutants, the authors started a continuously growing culture of Neurospora June 9, 1961. At present (October, 1962) this culture is still growing and being observed. The strain, ad-4; al-2 A has been continuously maintained at 30° C. on Vogel's medium supplemented with 0.2 mg. of adenine/ml. and 1.5% sucrose, and solidified with 1.5% agar. This strain has been grown in a continuous growth tube made up in sections each approximately 615 mm. long and 20 mm. in diameter. Each section is equipped with four sampling ports spaced along the top of the tube and 24/40 ground glass standard taper interchanges at each end. Plugs or closures made of the same type of ground glass joints are provided for each tube. The culture was inoculated at the proximal end of the growth tube. Before the culture reached the distal end of the tube, the distal closure was removed and a new tube joined to the first. Once the mycelium passed from the first section of the tube and was well established in the new section, the tubes were separated and a sterile closure used to seal the tube again. Before the proximal tube was discarded, a sample of the conidia from under each sampling port was removed, subcultured, and stored in a refrigerator for future analysis. The addition of new sections of growth tubes has permitted continuous hyphal propagation of the culture for 16 months with no need to subculture the organism.

The initial rate of growth of the culture for approximately 1,800 mm. averaged 5.8 mm./hr. Subsequently a progressive decline in growth rate was observed. In the next 12 sections of the growth tube (approximately 7,300 mm. of growth) the culture grew at a fairly constant rate of 3.5 mm./hr. This represents about three and a half months of total growth. In section XV the average rate of growth was 3.3 mm./hr. and in the proximal portion of section XVI the growth rate averaged 2.3 mm./hr. By the time the culture reached the distal end of section XVI, however, the growth rate had slowed to 0.9 mm./hr. and continued at this rate through succeeding sections up to section XXIII, where growth ceased. Since the growth rate had changed so drastically in section XVI, we were interested in determining whether the causative agent was already present but unexpressed in the proximal end of section XVI or

whether the event(s) responsible for the change had occurred between the proximal and distal ends. Consequently besides adding a new section of growth tube to the distal end, we also added another section to the proximal end of the tube. Since then two sets of growth tubes have been maintained.

Starting with section XXIII and in succeeding sections, a cycle of stopping and starting of the culture was observed. For example, in section XXIII growth ceased for 8 days and then resumed, again at a submaximal rate, averaging slightly less than 1 mm./hr. The culture again stopped in section XXIV for 10 days; in section XXV, for 15 days and again in the distal end of XXV for 22 days. In section XXVI it stopped for 5 days, grew an additional 105 mm. and then stopped again for 45 days, resumed growing for 240 mm. and again stopped for 32 days. At this writing (October 2, 1962), the culture has again resumed growth at 1.5 mm./hr.

On the other hand, the growth of the culture started from the proximal end of section XVI has been somewhat more consistent and has averaged approximately 2 mm./hr. compared with less than 1 mm./hr. for the culture in the original portion of the growth tube. The culture growing at 2 mm./hr. has stopped twice, once for 11 days and shortly after resuming growth again, for 27 days. It has grown at a fairly constant rate since then, although some periodic changes in growth rate have been observed.

Certain degenerative changes have been periodically observed in the morphology of both cultures. The ability to conidiate has been greatly impaired if not entirely lost, as has the ability to function normally as a perithecial parent in sexual crosses. Evidence suggests that the changes in growth rate are, at least in part, extranuclear. For example, growth in section XX averaged .82 mm./hr. Conidia from this section were removed and made into a heterokaryon with a compatible strain of the genotype pan-1; al-1 A. ad-4; al-2 A and pan-1; al-1 A homokaryons were then isolated from this heterokaryon and their growth rates determined. Such determinations showed that although some of the pan-1 al-1 A isolates maintained a growth rate comparable to the normal control culture from which the heterokaryon was prepared, it was also possible to isolate pan-1; al-1 A homokaryons whose growth rate was less than half that of the normal control. In a like manner certain of the ad-4; al-2 A homokaryons persisted at the much reduced growth rate; however, it was possible to obtain a significant number of ad-4; al-2 A isolates whose growth rates averaged 2.95 mm./hr. This is a significant increase in the rate of growth compared with the .82 mm./hr. of the adenine-requiring culture used to prepare the heterokaryon. It thus appears that a portion of the reduced growth rate of the ad-4; al-2 A culture can be overcome by what we colloquially refer to as rejuvenation by normal cytoplasm. In similar rejuvenation experiments, homokaryotic ad-4; al-2 A strains that produced abundant conidia were recovered; whereas, the culture in the growth tube from which they were derived had very few, if any, macroscopically visible conidia. Further investigation will determine, if possible, the nature of the observable changes found in the continuously growing culture. ---Department of Agronomy, Kansas State University, Manhatten, Kansas.