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Filamentous Growth of *Mucor rouxii* Under Nitrogen

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The form of growth of *Mucor rouxii* (National Regional Research Laboratory 1894) under nitrogen is dependent on inoculum size. With a large inoculum (10^6 spores inoculated per ml), the morphology consists mainly of swollen spores with some filaments and yeastlike cells. At lower inoculum levels growth is filamentous. The morphology of this strain on incubation under nitrogen and the dependence of the form of growth on inoculum size are similar to those found previously for other strains of *M. rouxii*.

Induction of purely yeastlike development in *Mucor rouxii* has been shown to result from incubation of sporangiospores anaerobically with a pCO_2 of 0.3 atm or higher (1); only filamentous growth was observed under nitrogen alone. Five strains of *M. rouxii* employed behaved similarly. On the other hand, one strain of *M. subtilissimus* [National Regional Research Laboratory (NRRL) 1909] grew in the yeastlike form under nitrogen alone. Haidle and Storck (4) reported that a strain of *M. rouxi* (NRRL 1894) grew in the yeastlike form when incubated under nitrogen and that aerobiosis was requisite for filamentous development.

Inoculum size was shown by Bartnicki-Garcia and Nickerson (2) to drastically affect morphology under nitrogen. With a large inoculum (3.6×10^5 spores per ml), a culture incubated for 24 hr under nitrogen consisted of spherical swollen spores of 18 μm average diameter. Because Haidle and Storck employed a very large inoculum (10^6 spores per ml), we have investigated the effect of inoculum size on morphological development in strain 1894 of *M. rouxii*.

MATERIALS AND METHODS

Mucor rouxii NRRL 1894 was obtained through the courtesy of C. W. Hesseltine (Northern Utilization Research and Development Division, Peoria, Ill.).

A defined medium supplemented with 0.2% vitamin-free casein hydrolysate was prepared as previously described (2). Inocula were obtained by harvesting spores from 7-day cultures on solid medium (yeast extract, 0.3%; peptone, 1.0% glucose, 2.0%; and agar, 3.0%). Sterile distilled water was added to each slant, and the sporangiospore-bearing turf was rubbed gently with a pipette; the dense spore suspension thus obtained was washed aseptically by centrifugation with three changes of sterile distilled water. The number of spores used as inoculum was determined by direct count with a hemacytometer.

During incubation, a nitrogen atmosphere was maintained by use of the arrangement shown schematically in Fig. 1. Nitrogen (prepurified grade, 99.997% purity) was purchased from the Matheson Co., Inc., East Rutherford, N.J. Trace amounts of oxygen in the nitrogen were removed by bubbling the gas through two columns of alkaline pyrogallol solution. Before inoculation, the incubation system was flushed continuously with nitrogen at a rate of 100 cm^3/min. The oxygen content of the exhaust gas was monitored by means of an oxygen probe coupled to a recorder. When the oxygen had been removed, the medium was inoculated. Samples could be taken with out disturbing the atmosphere of incubation by tipping the flask. Six of these shake-flask systems were arranged in parallel from the nitrogen source; only one flask was monitored for oxygen, however. Cultures were incubated at 25 C and agitated at 200 rev/min. The gas flow rate was maintained at 30 cm^3/min.

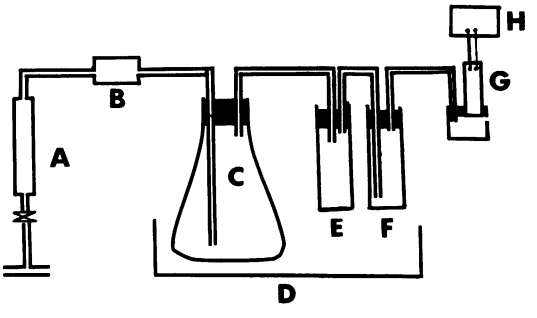

FIG. 1. *Apparatus used to maintain a nitrogen atmosphere of incubation. Letter designations: A, flow meter; B, filter; C, 300-ml Erlenmeyer flask containing 150 ml of medium; D, gyrotory water-bath shaker (New Brunswick Scientific Co., New Brunswick, N.J.); E, sample tube; F, water seal; G, oxygen probe (Precision Scientific Co., Chicago, Ill.); H, potentiometric recorder (Leeds and Northrup Co., Philadelphia, Pa.).*

RESULTS

The effect of inoculum size on the morphology of *M. rouxii* after incubation for 18 and 36 hr under nitrogen is shown in Fig. 2. Growth at 18 hr in the medium inoculated with 10^6 spores/ml consisted mostly of ungerminated spores and swollen spores, a few budding, yeastlike cells, and a few spores showing short germ tubes (Fig. 2A). Growth resulting from an inoculum of 10^5 spores/ml was mostly filamentous; only an occasional ungerminated spore was observed. The morphology of cells grown from an inoculum of 10^4

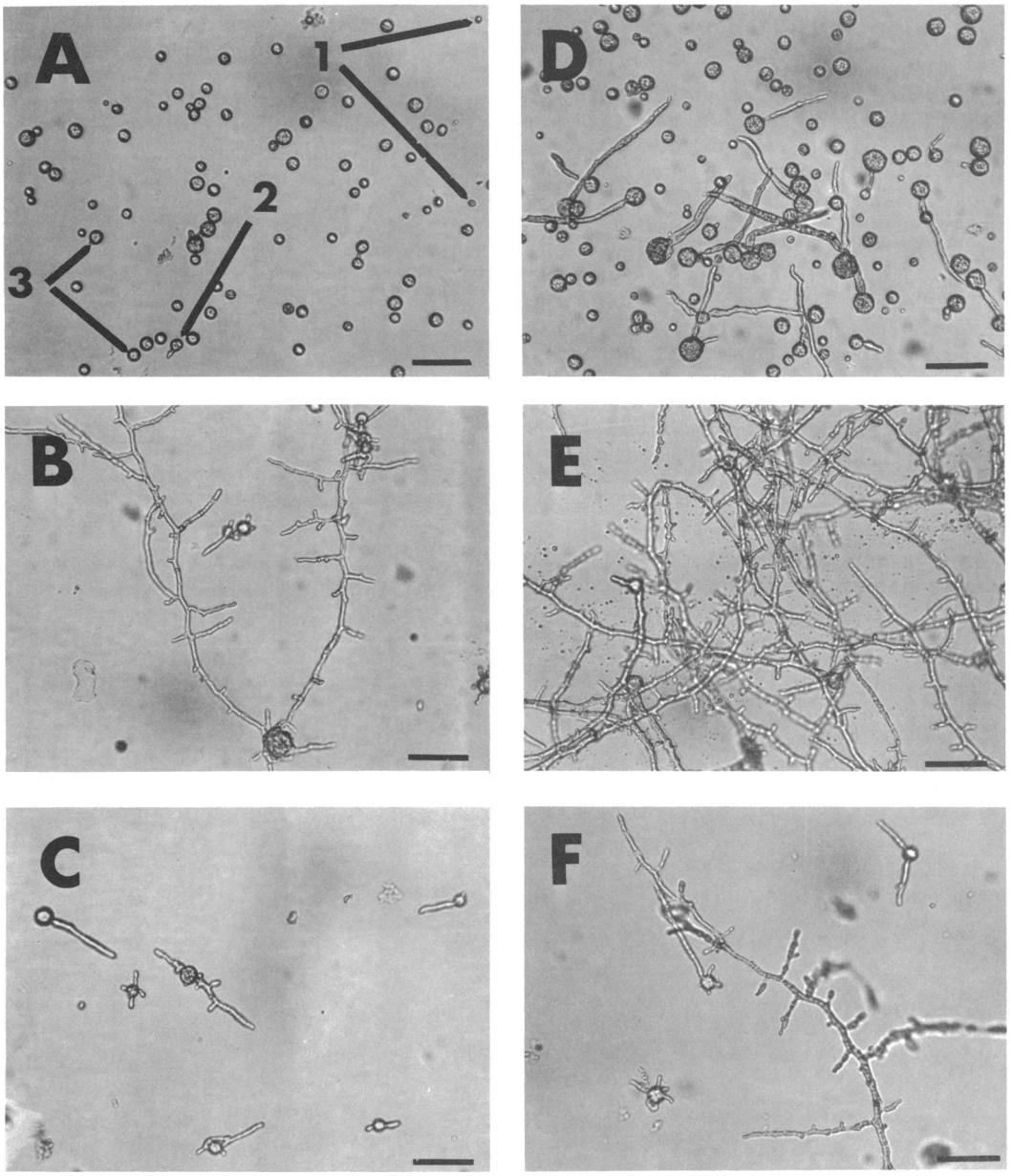

FIG. 2. *Effect of inoculum size on the morphology of M. rouxii incubated under N_2. Letter designations: A, growth at 18 hr with inoculum of 1×10^6 spores/ml showing ungerminated spores (1), a few spores with germ tubes (2), and many swollen spores (3); B and C growth at 18 hr with inocula of 10^5 and 10^4 spores/ml, respectively; D, E, and F, growth at 36 hr with inocula of 10^6, 10^5, and 10^4 spores/ml, respectively. Bar indicates 50 μm.*

spores/ml was entirely filamentous. After 36 hr, the effect of inoculum size on the morphology was less pronounced. Growth was filamentous except for the presence, in the medium receiving the largest spore inoculum, of some ungerminated spores, many swollen spores and, possibly, a few yeastlike cells.

DISCUSSION

The response of strain NRRL 1894 of *M. rouxii* on incubation under a nitrogen atmosphere is the same as that of five other strains previously studied. Growth under nitrogen is completely filamentous except when a very large inoculum is employed. Thus, aerobiosis is not necessary for filamentous development of this strain. With a very large inoculum, the morphology becomes mixed, consisting of filaments, a few budding yeastlike cells, together with some ungerminated spores and many swollen sporangiospores.

The effect of inoculum size on morphology is particularly pronounced during the initial 24-hr incubation period. The so-called "yeastlike" cells described by Haidle and Storck (4) were obtained from cultures incubated for 18 hr. Their illustration of "yeastlike cells" (Fig. 2; reference 4) reveals a population consisting largely of swollen spores with some ungerminated spores and few, if any, budding yeastlike cells. Spherical swollen spores and spherical arthospores may be mistaken for yeastlike cells, and it was for this reason that Bartnicki-Garcia and Nickerson (1) stated "it is necessary to emphasize that our definition of yeastlike growth of *Mucor* applies only to those cells which originate and multiply by budding." In fact, strain 1894 of *M. rouxii* does not develop exclusively in the yeastlike form in the basal medium except under 100% CO_2 in basal medium supplemented with substrate levels of small peptides (3). Thus, the contention by Haidle and Storck (4) that CO_2 does not play a specific role in promoting yeastlike growth of *M. rouxii* NRRL 1894 (and their implication that the same may hold for other strains of this species) cannot be supported by work from this laboratory.

ACKNOWLEDGMENT

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