INDUCTION OF YEASTLIKE DEVELOPMENT IN *MUCOR* BY CARBON DIOXIDE

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ABSTRACT

BARTNICKI-GARCIA, S. (Rutgers, The State University, New Brunswick, N. J.) AND WALTER J. NICKERSON. Induction of yeastlike development in *Mucor* by carbon dioxide. J. Bacteriol. **84**:829–840. 1962—Vegetative development of *Mucor rouxii* may follow either one of two patterns of morphogenesis (mold-yeast dimorphism), depending on the atmosphere of incubation. Under air or N_2, a filamentous (moldlike) growth developed, commonly followed by fragmentation of hyphae into spherical cells (arthrospores). Introduction of CO_2 into an anaerobic atmosphere induced development of spherical, budding yeastlike cells. Anaerobically, a pCO_2 of 0.3 atm or higher produced a purely yeastlike development. Presence of oxygen annulled the effect of CO_2 On germination, spores gave rise directly to either type of vegetative development, depending on the atmosphere of incubation. Induction of yeastlike development by CO_2 occurred in five strains of *M. rouxii*, and in most species of *Mucor* tested. *M. subtilissimus*, however, did not require CO_2; it developed in the yeastlike form under anaerobic conditions. Strains of *Rhizopus* grew under CO_2, but developed only filamentous mycelium. Members of other genera of *Mucorales* were unable to grow under an atmosphere of CO_2.

Among many fungi, vegetative development may follow different patterns of morphogenesis leading to formation of two drastically different morphological types, one filamentous and the other yeastlike. This duality in morphogenesis, commonly termed mold-yeast dimorphism, is subject to environmental control. A given pattern of development results from incubation under a specific set of environmental conditions, characteristic for a given fungus.

Mold-yeast dimorphism was discovered in a *Mucor* more than a century ago (Berkeley, 1838; Bail, 1857). At that time, the appearance of a fungus in two different morphological types was misinterpreted, and taken to represent a transmutation of species. Subsequent studies (Pasteur, 1876; Reess, 1870; Fitz, 1873) disproved this interpretation and clearly established the capacity of a single strain of *Mucor* to express different morphologies under different environmental conditions. Incubation of *Mucor* under aerobic conditions produced typical moldlike growth. Yeastlike growth appeared when cultures were insufficiently aerated. Yeastlike development was considered to be a response of the fungus to deprivation of oxygen. Brefeld's (1873) claim that accumulation of CO_2, rather than deprivation of oxygen, was actually responsible for yeastlike development of *Mucor racemosus* started a controversy as to the nature of the agent inducing yeastlike development. Klebs (1896) and Wehmer (1905) favored the view that absence of oxygen was the most important factor in yeastlike development of *Mucor*. Nadson and Philippov (1925), on the other hand, obtained purely yeastlike development of *M. guilliermondii* by incubation under CO_2.

Formation of spherical cells by *M. rouxii* has long been known to occur (Wehmer, 1900). Our studies of *M. rouxii* were undertaken to elucidate the biochemical bases of morphogenesis in the mold-yeast dimorphism. The work reported herein describes the behavior of *M. rouxii*, as well as several other members of the *Mucorales*, during cultivation under different atmospheres. Studies on the composition and structure of isolated cell walls of filamentous and yeastlike forms of *M. rouxii* are described elsewhere (Bartnicki-Garcia and Nickerson, 1962).

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TABLE 1. *Growth and morphogenesis of species of Mucor grown anaerobically in liquid YPG medium*

Organism	Strain*	Anaerobic atmosphere†			
		N_2		CO_2	
		Growth	Morphology	Growth	Morphology
M. rouxii	ATCC 4855	++	MF	+++	MY
M. rouxii	ATCC 5857	++	F	++	MY
M. rouxii	ATCC 8097	++	MF	+++	Y
M. rouxii	CBS	++	MF	++	MY
M. rouxii	IM 80	++	MF	+++	Y
M. subtilissimus	NRRL 1909	+++	Y	+++	Y
M. subtilissimus	NRRL 1743	++	MF	+++	Y
M. racemosus (+)	NRRL 1427	+	F	+	MF
M. racemosus (−)	NRRL 1428	++	MF	+	Y
M. rammanianus	IM 93	Not tested		0	—
M. mucedo	DC	Not tested		+++	MY
Mucor sp.	DU	Not tested		++	Y

* ATCC = American Type Culture Collection; CBS = Centraalbureau voor Schimmel-cultures; IM = Institute of Microbiology, Rutgers University; NRRL = Northern Regional Research Laboratory, cultures received through the courtesy of C. W. Hesseltine; DC = Douglass College, Rutgers University; DU = Duke University School of Medicine.

† In all cases, cultures incubated under air showed heavy filamentous growth, with little or no fragmentation. Amount of growth was estimated visually: 0 = no growth; +++ = abundant growth. Morphology of the cultures was classified as follows: F = purely filamentous; MF = mostly filamentous, with some spherical cells; Y = purely yeastlike; MY = mostly yeastlike, with few filaments.

MATERIALS AND METHODS

M. rouxii (Calmette) Wehmer, strain IM 80 from the Culture Collection of the Institute of Microbiology, was used. Origins of other microorganisms employed are mentioned in Tables 1 and 2.

A complex culture medium (YPG) of the following composition was employed: Difco yeast extract, 3 g; Difco peptone, 10 g; glucose, 20 g; and distilled water to make one liter; pH was adjusted to 4.5 with H_2SO_4. The medium was delivered in 50-ml quantities into Erlenmeyer flasks and autoclaved at 121 C for 15 min. To prepare solid YPG medium, 30 g of agar were added per liter of liquid YPG medium prior to autoclaving. The sterilized medium was then poured in 10-ml amounts into 20 × 100 mm petri dishes.

Inocula were prepared from sporulated slant cultures. A few ml of sterile water were added to each slant; the growth was rubbed with a sterile pipette, and the spore suspension obtained was used directly as inoculum.

Liquid cultures were incubated on a reciprocating shaker at 28 C for 48 hr. To control the atmosphere of incubation, rubber stoppers with inlet and outlet tubing connections were fitted to the flasks. Duplicate flasks were connected in series, and a bubble counter was provided to regulate flow rate (Fig. 1). Gases were flushed through the gas space of flasks, at an approximate rate of three bubbles per sec, during the entire incubation period. A Gast portable rotary air blast apparatus was utilized to provide a stream of air. Prepurified N_2 of 99.996% purity, and bone-dry grade CO_2 of 99.8% purity, were obtained from the Matheson Co., Inc., East Rutherford, N. J. Gas mixtures of the above gases were prepared in a 75-liter gas holder (A. H. Thomas, Co., Philadelphia, Pa.) with a final pressure of 1 atm.

Anaerobic incubation of cultures on solid media was performed in a desiccator. Air was replaced with either N_2 or CO_2 by repeated evacuation and filling (five times). When a N_2 atmosphere was used, a dish containing 20% KOH solution was introduced to absorb metabolically generated CO_2.

Growth was measured according to the following procedure. Each flask was vigorously shaken and the contents filtered through a double layer of cotton gauze (27 mesh). Growth retained by

TABLE 2. *Growth and morphogenesis in representatives of various genera of Mucorales grown under air or CO_2 in liquid YPG medium*

Organism	Strain*	Atmosphere†			
		Air		CO_2	
		Growth	Morphology	Growth	Morphology
Rhizopus oryzae	DU	+++	F	++	F
R. arrhizus	DU	+++	F	+	F
R. fusiformis	CBS	+++	F	+	F
R. nigricans	IM 85	+++	F	+	F
Rhizopus sp.	DU	+++	F	+	F
Rhizopus sp.	DC	+++	F	0	—
Phycomyces blakesleeanus	IM 91	++	F	0	—
Zygorhynchus sp.	PP	+++	F	0	—
Circinella sp.	PP	+	F	0	—
Mortierella sp.	PP	+++	F	0	—
Mortierella sp.	PP	+++	F	0	—
Sporodinia sp.	PP	+++	F	0	—
Cunninghamella verticillata	ATCC 8983	+++	F	0	—

* Origin of the strains given in Table 1; in addition: PP = Plant Pathology Department, Rutgers University.

† Growth and morphology of the cultures was classified as stated in Table 1.

the gauze was composed of long branched filaments and constituted the "filamentous fraction." Growth passing through consisted of variable proportions of single spherical or ellipsoidal cells, short cellular chains, and short filaments. This was termed the "fragmentary fraction" of growth. To remove the filamentous fraction from gauze, the latter was inverted, stretched over a 250-ml beaker, and washed with a strong jet of distilled water. Dry weight of each fraction was determined by filtration through a Pyrex fritted-glass crucible, porosity M, followed by extensive washing with distilled water and desiccation at 80 to 90 C for 24 hr. Total growth of a flask was calculated as the sum of fragmentary and filamentous fractions.

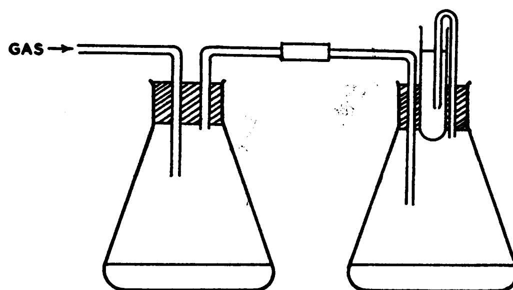
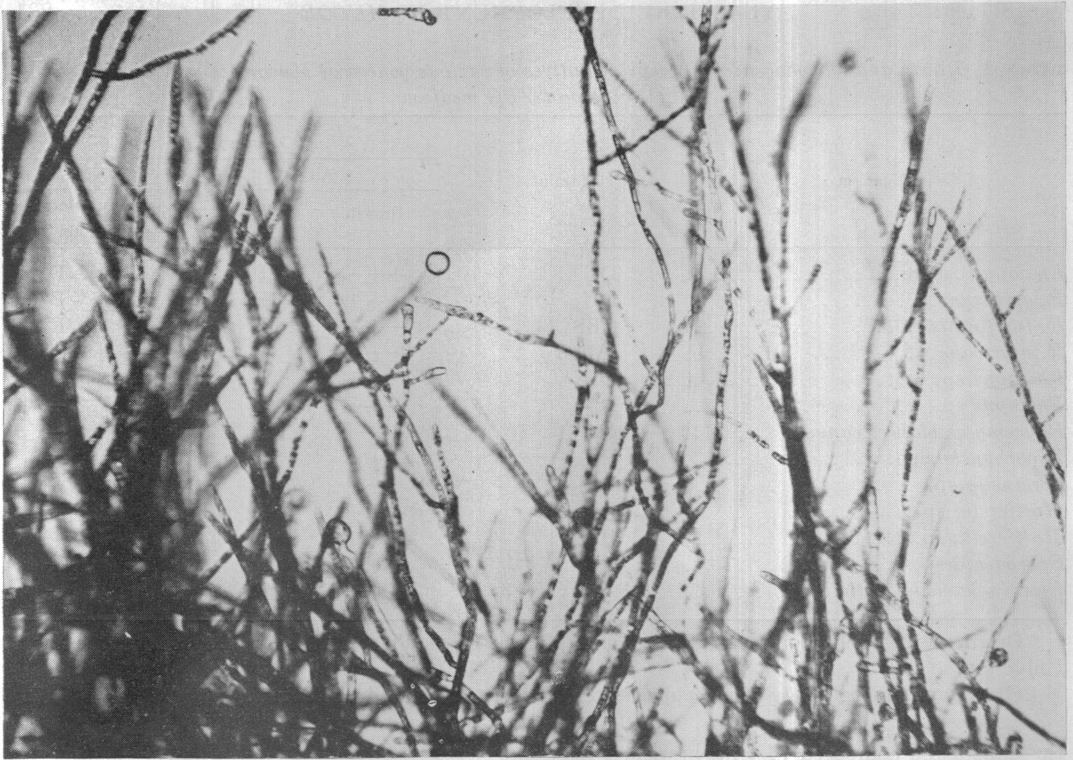


FIG. 1. *Arrangement employed for incubation under controlled atmosphere. Flasks mounted on a reciprocating shaker with flexible coupling to gas manifold.*

RESULTS

Atmospheric control of mold-yeast dimorphism in M. rouxii. Aerobic incubation of *M. rouxii* under continuous agitation resulted in rapid, abundant moldlike growth (Fig. 2, top). Initially, growth consisted of a finely dispersed suspension of filaments, which later developed into small filamentous pellets. After 24 hr of incubation, these pellets had become large entangled masses of filamentous mycelium. This filamentous growth, typical of *Mucorales*, consisted of coenocytic mycelium with hyphae of variable width. During the first 12 hr of incubation, cultures exhibited a purely filamentous morphology (Fig. 3A and 3B). Thereafter, a small proportion of spherical cells began to appear. These cells did not originate by budding, but by arthrospore formation, a process of hyphal fragmentation which commenced with formation of septa at or near the tips of filaments (Fig. 3C). Segments of hyphae walled off by the nonperforated septa increased in volume and acquired a spherical shape (Fig. 3D and 3E). Individual arthrospores, or chains thereof, were easily detached from the hyphae, especially as arthrospores approached



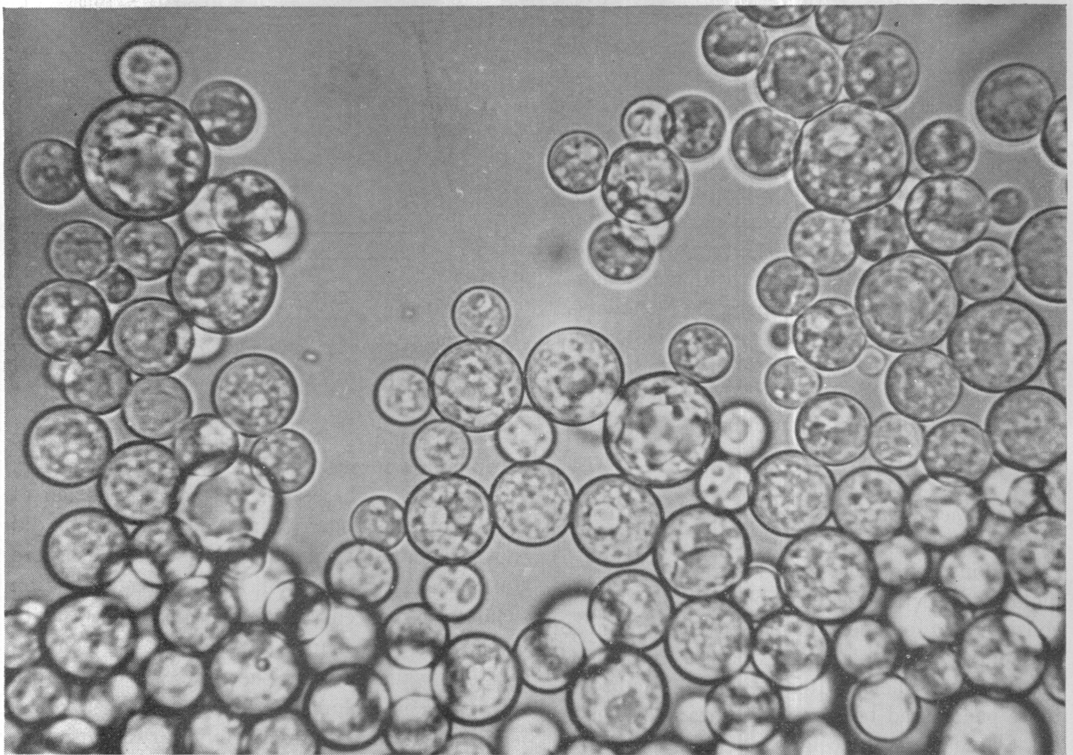


FIG. 2. *Dimorphism of Mucor rouxii. Filamentous form grown under air (top). Yeastlike form grown under* CO_2 *(bottom). Stain: dilute Lugol's solution. Magnification: 125 × and 625 ×, respectively.*

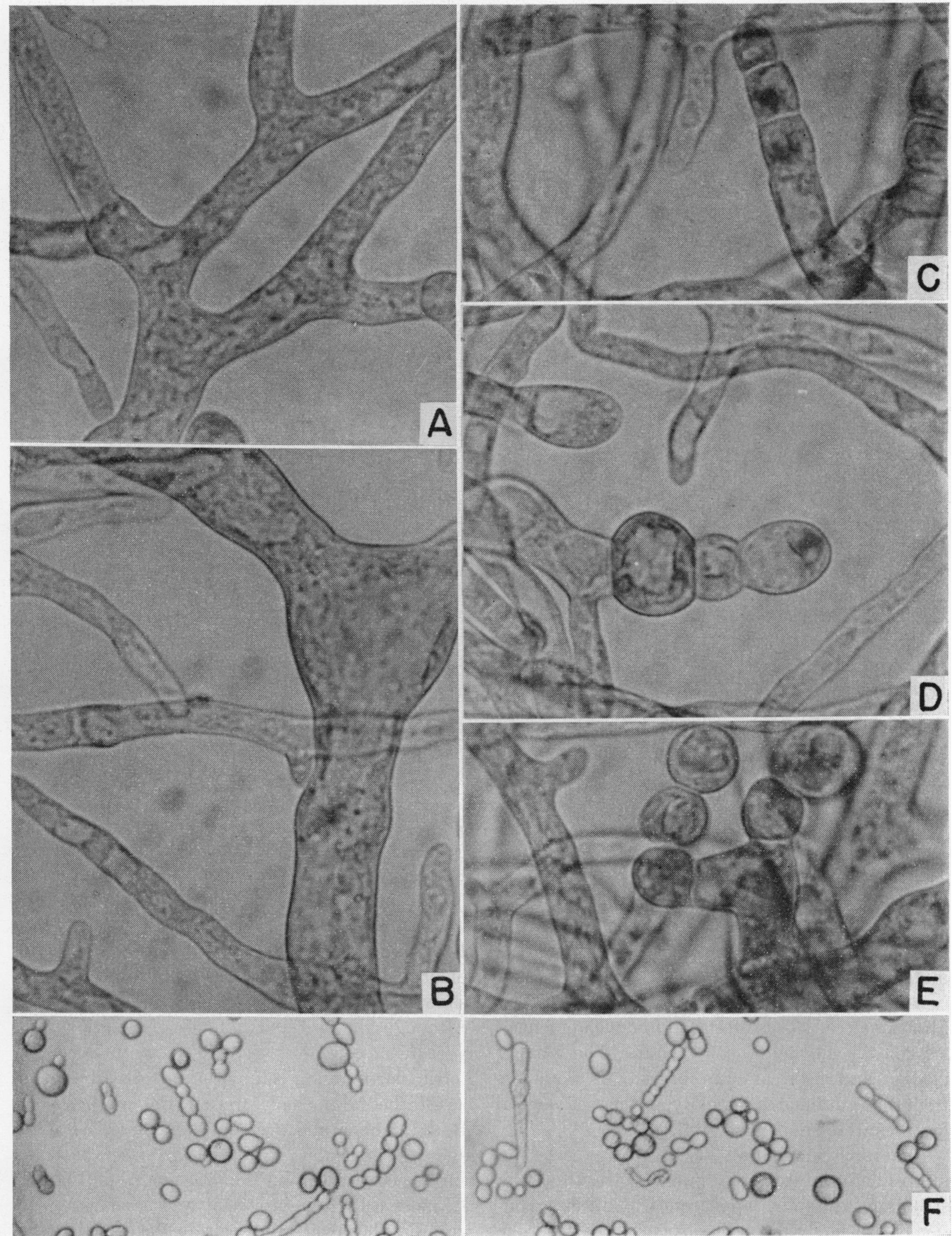


FIG. 3. *Aerobic filamentous form of Mucor rouxii. Young coenocytic hyphae* (A, B); *progressive stages in arthrospore development* (C, D, E); *fragmentary growth obtained after 48 hr of incubation* (F). *Stain: dilute Lugol's solution. Magnification: A-E, 900 ×; F, 225 ×.*

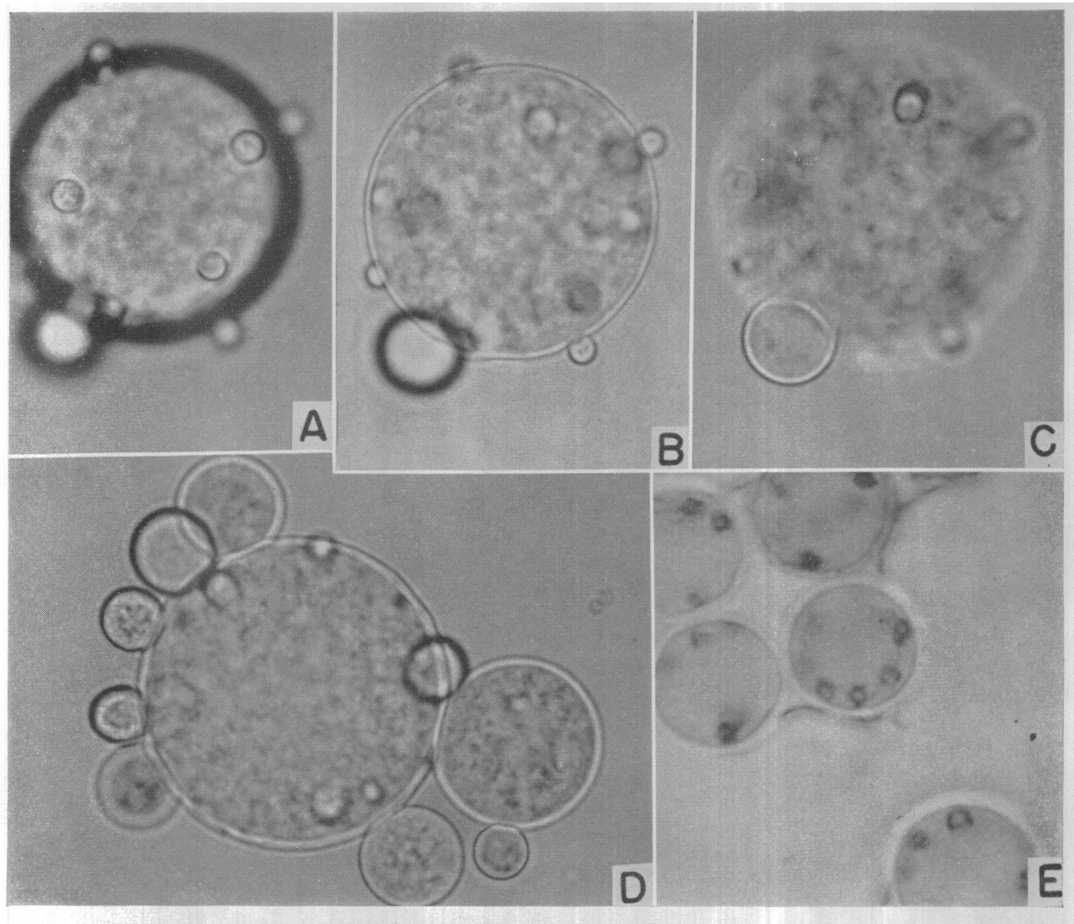


FIG. 4. *Yeastlike cells of Mucor rouxii. Distribution of buds around mother cell as shown by optical sectioning from top* (A), *through equator* (B), *to bottom* (C); *vertical travel* = *23 μ; magnification: 1,325 ×. Multipolar budding cell* (D); *magnification: 1325×. Nuclei stained with HCl-Giemsa* (E); *magnification: 900 ×.*

sphericity and their zone of adherence was minimized (Fig. 3F).

Anaerobic incubation of *M. rouxii* under an atmosphere of N_2 also resulted in development of a typically filamentous mycelium. The rate of growth and total amount of growth were less than that obtained aerobically, but morphological features were similar.

Anaerobic incubation of *M. rouxii* under an atmosphere of essentially pure CO_2 resulted in a strikingly different appearance. Cultures consisted of a finely disperse suspension of yeastlike cells which sedimented rapidly upon standing. Microscopy revealed the presence of yeastlike cells with no trace of filamentation (Fig. 2, bottom). Yeastlike cells are spherical bodies of 10 to 50 μ diameter; larger cells were found in older cultures. A variable proportion of cells showed characteristic multipolar spherical buds. Although the size and number of buds per cell varied, a common yeastlike structure was a tetrapolar budding cell, with buds distributed in a tetrahedral fashion around the mother cell. Both number and size of buds increased with age; a conspicuous structure was a cell with its surface covered by buds of various sizes (Fig. 4). In most cells the cytoplasm was filled with fine granules; however, some cells in the same culture exhibited a coarse granulation. By the HCl-Giemsa technique of Robinow (1957), yeastlike cells were shown to be multinucleate. Young cultures (8 to 12 hr) exhibited six to eight nuclei

per cell (Fig. 4E), but the number increased as the cells aged and enlarged.

Influence of pCO_2 on morphogenesis of M. rouxii. Since incubation under CO_2 determined yeastlike development of *M. rouxii*, the influence of partial pressure of carbon dioxide (pCO_2) on growth and morphogenesis was evaluated. Aerobic and anaerobic atmospheres were adjusted to various pCO_2 values, and the resulting filamentous and fragmentary growth fractions were estimated. The fragmentary fraction may not consist exclusively of yeastlike growth, but may be composed partly or entirely of small segments of hyphae, chains of spherical cells, and individual spherical cells, all of which originated by arthrosporal morphogenesis. However, comparison of values for fragmentary growth with microscopic appearance provided a basis for estimating the fraction of growth resulting from either yeastlike or arthrosporal development.

Figures 5 and 6 illustrate growth responses after 24 hr of incubation under aerobic and anaerobic conditions, respectively. In aerobic atmospheres, increase in pCO_2 led to a sharp decrease in the amount of growth due, possibly to a major extent, to deprivation of oxygen. Aerobic growth was always more abundant than anaerobic growth. On comparing values for growth under anaerobic atmospheres (Fig. 6) with growth under 1 atm of air (Fig. 5), one obtains ratios for aerobic:anaerobic growth which range from 2.6 to 5.9, depending on the pCO_2 of the anaerobic atmosphere. A pCO_2 of 1 atm was partly inhibitory for growth, but pCO_2

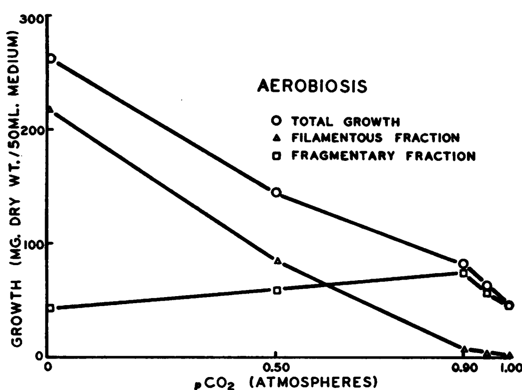


FIG. 5. *Effect of pCO_2 on growth and morphogenesis of Mucor rouxii incubated (24 hr) under mixtures of CO_2 and air at a total pressure of 1.0 atm.*

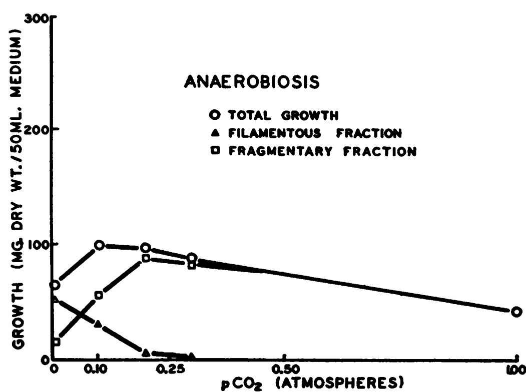


FIG. 6. *Effect of pCO_2 on growth and morphogenesis of Mucor rouxii incubated (24 hr) under mixtures of CO_2 and N_2 at a total pressure of 1.0 atm.*

values between 0.1 and 0.3 atm were stimulatory for anaerobic growth as determined after 24 hr of incubation.

Under either aerobic or anaerobic atmospheres, carbon dioxide caused filamentous growth to decrease significantly. As the pCO_2 increased, the length of filamentous cells decreased. Concurrently, the fragmentary fraction of the total growth increased, accompanied by a change in morphology of structures composing the fragmentary growth. Small filaments, and arthrospores in various stages of development, were gradually replaced by typical budding yeastlike spheres. Consequently, increases in amount of the fragmentary growth fraction evaluate the tendency towards yeastlike development (Fig. 5 and 6). Under anaerobiosis, a pCO_2 of about 0.3 atm sufficed to induce a purely yeastlike development. Aerobically, such a pCO_2 value produced chiefly filamentous growth with little evidence of budding in the fragmentary growth fraction; in the presence of oxygen, higher tensions of CO_2 (0.9 atm) were required to obtain a distinctly yeastlike appearance. Thus, small amounts of oxygen prevented the development of a purely yeastlike morphogenesis.

Even under atmospheres without added CO_2, and in flasks containing a central well with concd KOH to eliminate metabolic CO_2, the fragmentary growth fraction of typically filamentous cultures exhibited a small but significant proportion of spherical cells. These cells, however, were not yeastlike but arthrospores, derived by hyphal fragmentation. Hence, cultures incubated

in the absence of CO_2 were free of yeastlike morphogenesis, despite the presence of spherical cells.

Morphogenesis of M. rouxii on solid media. The effect of atmosphere of incubation on morphogenesis of *M. rouxii*, as described above for liquid cultures, was expressed similarly on solid YPG medium. Plates streaked with spores and incubated under air showed a rapidly growing and spreading filamentous turf (Fig. 7) which, after 24 to 48 hr, covered the entire surface of the plate. Numerous sporangiophores and sporangia were formed. The turf of aerial growth reached a thickness of about 1 cm. Microscopically, vegetative mycelium consisted of long, branched filaments with some arthrospores in various developmental stages.

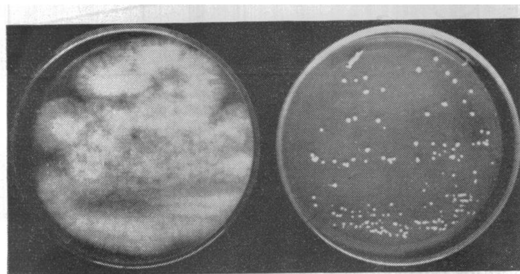


FIG. 7. *Development of Mucor rouxii on solid YPG medium. Incubation under air for 24 hr (left); incubation under CO_2 for 72 hr (right).*

In plates incubated under N_2, the fungus formed a thin film of slowly growing filamentous mycelium, which covered the entire plate in about 1 week. Microscopically, growth consisted of filaments with some arthrospores in various stages of development; no sporangia were seen.

Plates incubated under CO_2 exhibited characteristic yeastlike colonies (Fig. 7). After 72 hr of incubation, colonies were 2 to 3 mm in diameter, opaque, cream-colored, and convex, with an irregular surface and undulate border. There was no tendency for the fungus to spread. Colonies consisted entirely of yeastlike cells with multipolar buds and were indistinguishable from cells obtained in liquid culture. No sporangia or

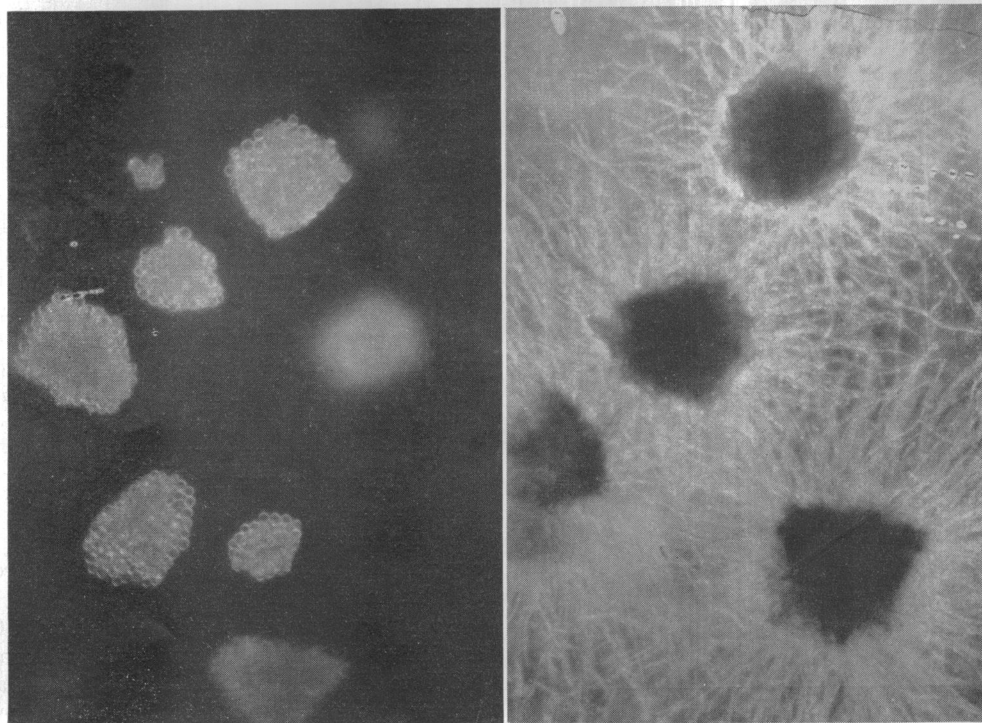


FIG. 8. *(left) Microcolonies of yeastlike cells of Mucor rouxii obtained by incubation of spores under CO_2 for 24 hr; magnification: 70 ×.*

FIG. 9. (right) *Microcolonies of plate illustrated in Fig. 8, after exposure to air for 8 hr; magnification: 70 ×.*

sporangiophores were formed. Occasionally, small filaments were seen arising from the borders of the colonies.

Mold-yeast dimorphism: a phenotypic duality. Experiments were made to answer the question: "Is mold- or yeastlike development of *M. rouxii* due to a heterogenous population in which two genetically different types are present—one capable of filamentous development, the other of yeastlike morphogenesis?" Spores derived from a single spore isolate of *M. rouxii* were plated on YPG agar and incubated under an atmosphere of CO_2. After 24 hr, only micro-colonies of yeastlike cells were visible (Fig. 8). A total of 5,000 such microcolonies were then exposed to air for 8 hr; whereupon, all of the microcolonies developed abundant filamentous growth (Fig. 9). The capability of all yeastlike cells to form filaments, immediately upon exposure to air, indicates that mold-yeast dimorphism of *M. rouxii* results from differences in phenotypic expression of the same genotype, rather than from any heterogeneity in the population.

Mechanics of spore germination in M. rouxii. Depending on the atmosphere of incubation, a spore of *M. rouxii* may develop, upon germination, into a hyphal tube or a spherical bud. The sequence of morphological changes occurring during either morphogenesis was followed by interval photography of individual spores germinating on a thin layer of YPG agar in a Porter tissue-culture flask. For aerobic germination, flasks were left unplugged. For germination under CO_2, flasks were fitted with rubber stoppers and tubing connections. Air was replaced with CO_2 by repeated evacuation and filling. The fundamental differences in morphogenesis during germination under air vs. CO_2 are compared in Fig. 10. Germination under air was relatively rapid; in about 3 hr a germ tube began to be formed. Under CO_2, onset of budding did not take place until after 12 hr. Under either atmosphere, germination began with swelling and rounding off of the originally elliptical spores, which enlarged from an average size of $4.5 \times 5.5\ \mu$ into spherical cells 10 to 15 μ in diameter. The swollen spore would then develop either a cylindrical germination tube or a spherical bud, depending on the atmosphere of incubation. Aerobically, a large area of the surface of the swollen spore bulged out, and a broad tube with a conical tip

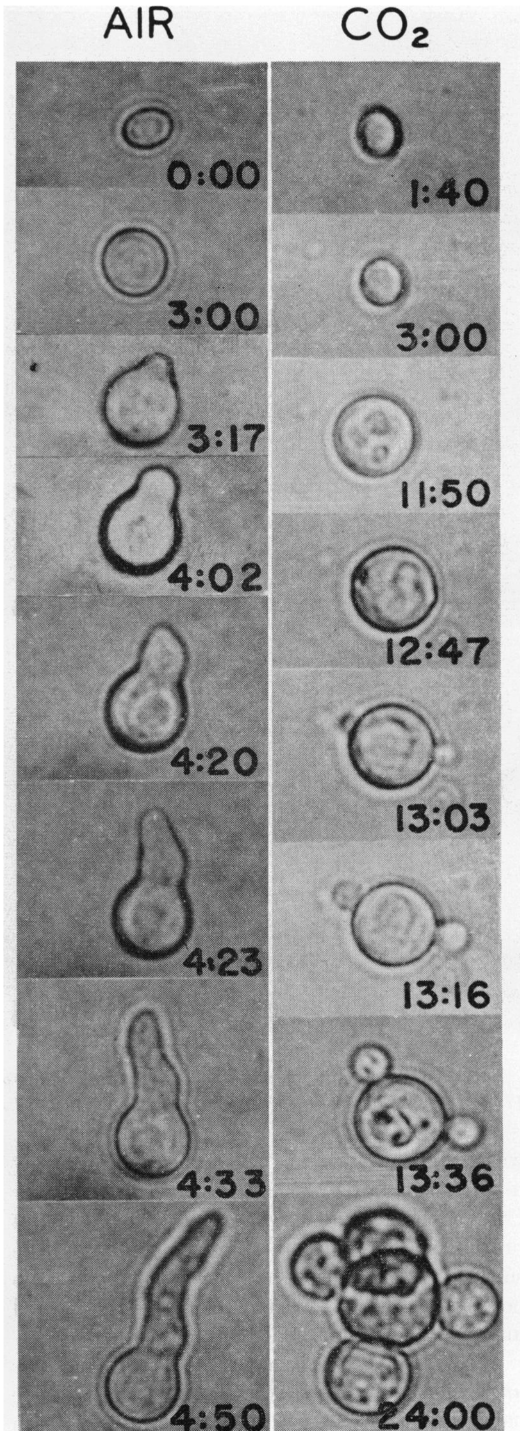


FIG. 10. *Germination of spores of Mucor rouxii under air or under CO_2; incubation time indicated in hr: min; magnification: 900 ×.*

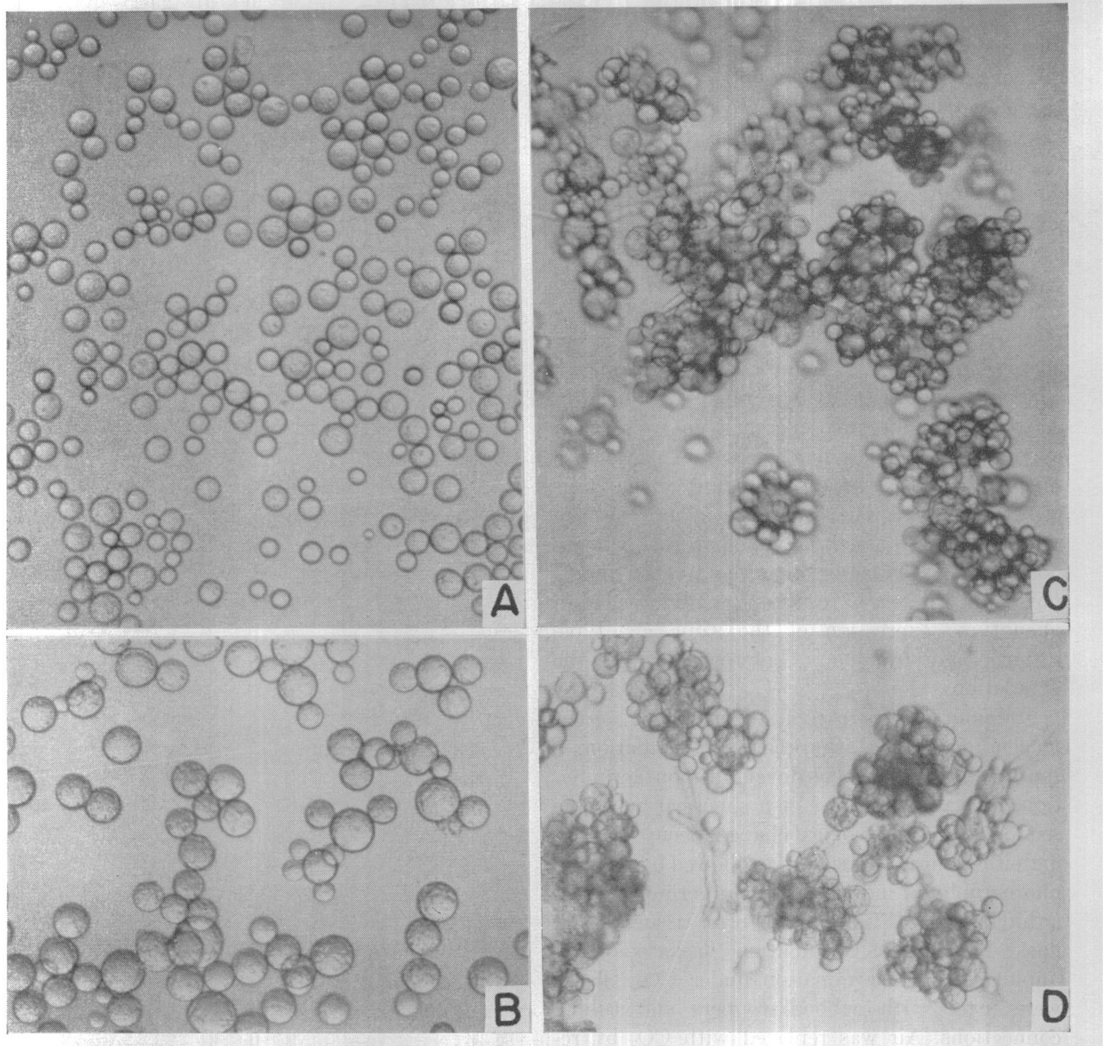


FIG. 11. *Yeastlike forms of species of Mucor. M. subtilissimus NRRL, 1909* (A); *M. subtilissimus NRRL 1743* (B); *M. rouxii CBS* (C)*; and M. rouxii ATCC 4855* (D)*; magnification: 225 ×.*

was formed. Growth of this tube was primarily unidirectional and, therefore, polarized. Under CO_2, spherical buds were formed by extrusion of protoplasm through a relatively small area of the surface of the swollen spore. Seemingly, no polarity of growth existed, since buds enlarged uniformly and approached a spherical shape.

Development of sporangia from yeastlike cells under inanition. Yeastlike cells of *M. rouxii* were harvested after 48 hr of incubation under CO_2 in YPG medium, then washed free of nutrients with a 0.3% aqueous solution of K_2HPO_4, and finally suspended in a shallow layer of 0.3% K_2HPO_4 in a petri dish. When incubated aerobically, the cells developed into long thin sporangiophores which projected above the liquid surface and bore terminal sporangia. Cells of the same suspension incubated anaerobically showed no development.

Mold-yeast dimorphism in Mucorales. Cultures of several species of *Mucor* were incubated under air, N_2, or CO_2 to survey the influence of incubation atmosphere on morphogenesis. Table 1 summarizes the results obtained. Four strains of *M. rouxii* behaved analogously to strain IM 80 previously described. They all developed chiefly,

or entirely, as yeastlike cells under CO_2, or as filaments under either air or N_2. Similarly, CO_2 induced yeastlike development in a strain of *M. subtilissimus* (NRRL-1743) and a strain (−) of *M. racemosus*. A strain (+) of *M. racemosus* grew very poorly and formed filamentous hyphae under CO_2. The one strain of *M. rammanianus* tested did not grow under CO_2. One strain of *M. subtilissimus* (NRRL 1909) deserves special mention since it developed exclusively yeastlike cells when incubated either under N_2 or CO_2. Apparently, CO_2 was not necessary for yeastlike development in this strain. Experiments made under more rigorous conditions of CO_2 exclusion (with KOH in a center well inside a culture flask) also resulted in yeastlike development, thus confirming the lack of an exogenous CO_2 requirement for yeastlike morphogenesis in this strain of *M. subtilissimus*. In microscopic appearance, yeastlike cells of *Mucor* varied in cell size and in number of buds attached to mother cells (Fig. 11). In *M. subtilissimus*, virtually all buds detached readily from the mother cells; in *M. rouxii* (strains ATCC 4855 and 4857), buds remained attached to the mother cell, resulting in large bunches of spherical cells. In some species of *Mucor*, incubation under CO_2 completely prevented filamentation; in others, some small filaments were seen (Table 1 and Fig. 11).

Members of other genera of *Mucorales* were incubated under air and under CO_2. Results summarized in Table 2 indicate that strains of *Rhizopus* were also capable of growing under an atmosphere of CO_2, but developed only filamentous mycelium with no indication of yeastlike development.

DISCUSSION

Vegetative development of *M. rouxii* may follow three main patterns of morphogenesis: filamentous, arthrosporal, or yeastlike. The first and second are consecutive and constitute what can be called the filamentous-arthrosporal cycle of development. The relationships among the three patterns of development are depicted in Fig. 12. A spherical cell is the common denominator for all three courses of vegetative morphogenesis.

Development of spherical cells by *M. rouxii* occurs through two basically different mechanisms: (i) yeastlike morphogenesis, induced by anaerobic incubation in the presence of CO_2, and (ii) arthrospore formation, which does not require either CO_2 or anaerobic conditions, and which appears to be a consequence of filamentous development.

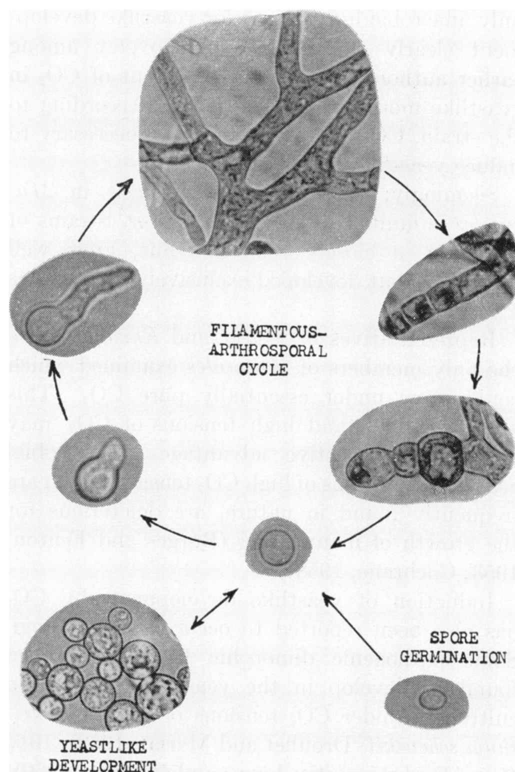


FIG. 12. *Patterns of vegetative morphogenesis in Mucor rouxii.*

Mold-yeast dimorphism contrasts formation of spherical cells by yeastlike morphogenesis with filamentous development via the filamentous-arthrosporal cycle. Spherical cells derived by arthrosporal morphogenesis must not be confused with truly yeastlike cells which, microscopically, may be indistinguishable from spherical arthrospores. It is necessary to emphasize that our definition of yeastlike growth of *Mucor* applies only to those cells which originate and multiply by budding.

For most species of *Mucor*, induction of yeastlike development involves two gaseous factors: presence of carbon dioxide and absence of oxygen. From our results with *M. rouxii*, it appears that oxygen abolishes the yeastlike inducing properties of CO_2. The fact that *M. subtilissimus* (strain NRRL 1909) does not require CO_2 (but

only anaerobic incubation) for yeastlike development clearly explains the controversy among earlier authors as to the involvement of CO_2 in yeastlike morphogenesis of *Mucor*. According to the strain, CO_2 may or may not be necessary to induce yeastlike development.

Seemingly, yeastlike morphogenesis in *Mucorales* is limited to species of *Mucor*. Strains of *Rhizopus*, a closely related genus, grew well under CO_2 but developed exclusively filamentous mycelium.

Representatives of *Mucor* and *Rhizopus* were the only members of *Mucorales* examined which could grow under essentially pure CO_2. This ability to withstand high tensions of CO_2 may represent a selective advantage in microbial ecology; conditions of high CO_2 tension, which are frequently found in nature, are deleterious for the growth of many fungi (Burges and Fenton, 1953; Cochrane, 1958).

Induction of yeastlike development by CO_2 has also been reported to occur in other fungi. Some pathogenic dimorphic fungi have been found to develop in the yeastlike form when cultivated under CO_2 tensions of 5% (*Sporotrichum schenckii*; Drouhet and Mariat, 1952); 10% (*Coccidioides immitis*; Lones and Peacock, 1960); and 15 to 20% (*Histoplasma farciminosus* and *H. capsulatum*; Bullen, 1949). In all of these cases, a major divergency from *Mucor* is the fact that oxygen was not inhibitory for CO_2 induction of yeastlike development (since the above CO_2 tensions were adjusted in air).

The observation that yeastlike cells can develop into sporangiophores and sporangia in nutrient-free solutions exposes the possibility of studying morphogenesis of sporulation without the complication of concomitant growth activities—a favorable experimental situation, equivalent to that encountered in the morphogenesis of cellular slime molds (Bonner, 1959).

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