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Full Length Research Paper

Sporangiospore-to-yeast conversion: Model for morphogenesis

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This report reviewed the specific characteristics of *Mucor circinelloides* in solid cultures and its capacity for multiple anamorphic expressions in synthetic broth, including holoblastic, holothallic, enterothallic conidia, and vesicular conidia head group as well as yeast cells. Attempt was made to show that the sequence of events in the conversion process, with inherent cytosolic nucleation and protoplast formation, to terminal budding yeast cell would serve as a model for studying morphogenetic transformation in the fungi.

Key words*: Mucor circinelloides,* conversion model, synthetic broth, sporangiospores, anamorphs, yeast cells.

INTRODUCTION

In 1996 a procedure was described (Omoifo, 1996) for obtaining terminal budding yeast cells from sporangiospores of filamentous microorganisms. Such induced morphology is different from the well known multipolar budding yeast like form to which *Mucor* species are generally converted in modified environments. The recent work by Lubberhusen et al. (2003) demonstrated the occurrence of *Mucor circinelloides* Tieghem as existing under varying conditions and through its life cycle as a central mother cell with different loci from which emerged globose daughter buds.

Since the work of Bartnicki-Garcia and Nickerson (1961, 1962a,b,c,d), some time and effort have been devoted to understanding the biochemical basis of such conversions. The review of Inderlied et al. (1985) and Ruiz-Hereiera (1985) as well as that by Bartnicki-Garcia and Gierz (1991) are good reference texts in the fur-ther attempt to underscore biochemical and molecular approaches to this study. Despite this effort, cellular differentiation during morphogenetic conversion is still far from being understood. Effort in this direction will depend largely on finding adequate model system for growth of the organism. The system should be simple chemical composition so as to facilitate easy manipulation and understanding of inherent biochemical reactions with predictable physiological and, or phenotypic outcome. These will occur in identifiable structures differing in sizes occupying specific space with definite shapes and recognizable at the simplest resolution, the light microscope. It is also valuable that such structures are restricted to specific phases in defining the life pattern.

In this way it will be possible to monitor the process of transformation that leads to the induction of specific anamorph of the microorganism. In this wise, several studies have emerged showing that *M. circinelloides* is capable of polymorphic existence (Omoifo, 2005, 2006ab; Omoifo and Omamor, 2005; Omoifo et al., 2006). The following is an attempt to show that *M. circinelloides* meets the conditions outlined above in its transformation to terminal budding yeast cells in clearly defined chemical medium.

VEGETATIVE GROWTH

In solid cultures of glucose-yeast extract-peptone agar initial growth of sporangiospores of *M. circinelloides* is isotropic but soon produce germ tube initial. This is followed by apical extensions; the coenocytic hyphal growth is rapid and luxuriantly produced thus interweaving to form a meshwork, as occurs in a colony (Figure 1).

Copious aerial sporangiophores are produced; these terminate with collumelae (Figure 2), which along with orange-coloured sporangiospores are enclosed within smooth sporangial walls (Figure 2). Numerous lateral sporangiophores also emerge that add to give the culture a compact appearance (Figure 3). Two types of sporangiophores-sporophores are observed: straight slightly

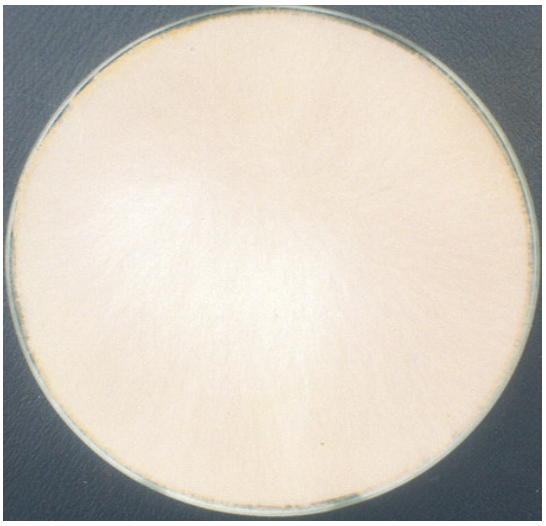

Figure 1. A-7 day old culture of *M. circinelloinelloides*.

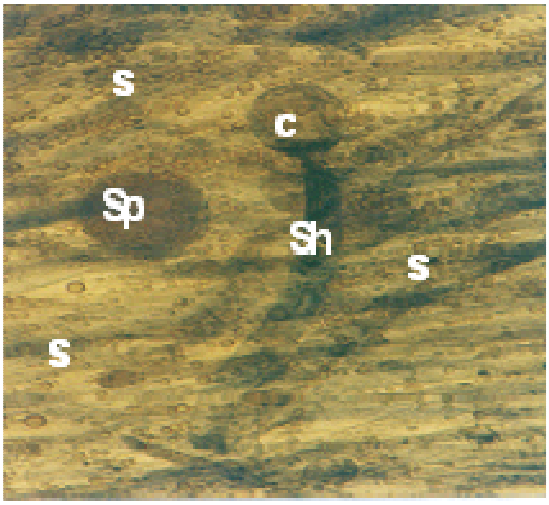

Figure 2. Sporangiospores (S), sporangiophore (sh), sporanngium (sp) and collumelum (c) of *M. circinelloides* cultivated in GYPA.

bent (Figure 2) and circinate (Figure 4). Also there are two types of collumelae: spherical (Figure 5) and pyriform, which ends with a truncate base (Figure 6). Lateral sporangia may be sessile, in which case they are borne directly on what appears to be a bough of substrate level hyphae or may have short sporosphores. In any case, lateral sporophores are not as long as the aerial types.

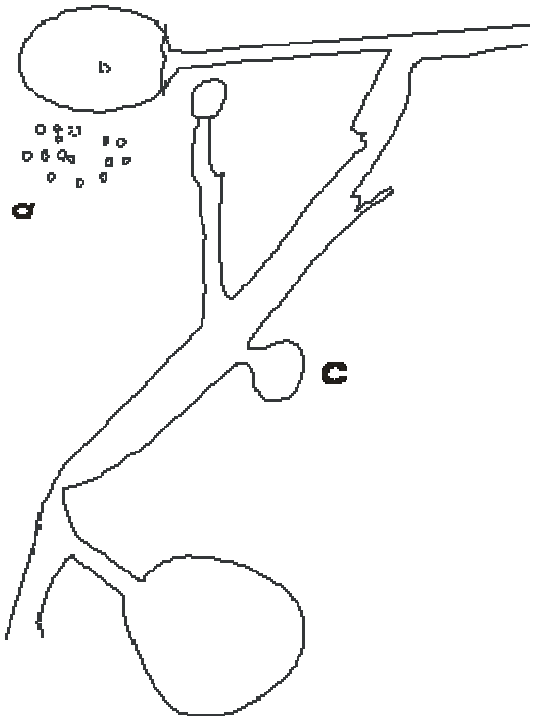

Figure 3. Diagram illustrating mycelium of *M. circinelloides*; a, Sporangiospores; b, globose collumelum with colarrette; c, lateral Sporangium.

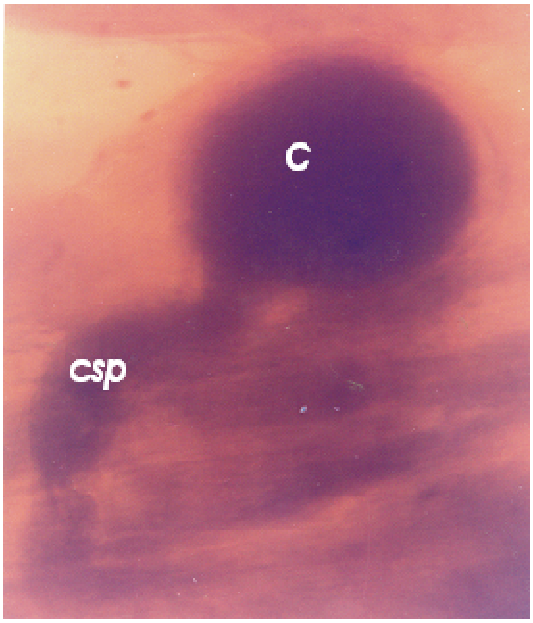

Figure 4. Circinate sporangiophore of *M. circinelloides* cultivated in GYPA.

FUNGAL DIMORPHISM

An alternative form of growth of *M. circinelloides* will entail the exhibition of the phenomenon of fungal dimorphism. The fate of sporangiospores of this Zygomycete in broth has been studied. Aerobically produced spores

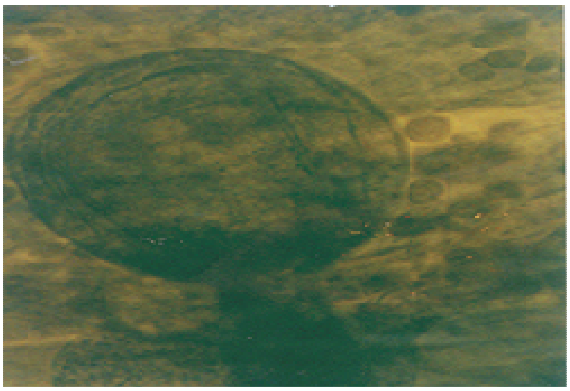

Figure 5. Spherical collumelum of *M. circinelloides* cultivated in GYPA; x2000 mag.

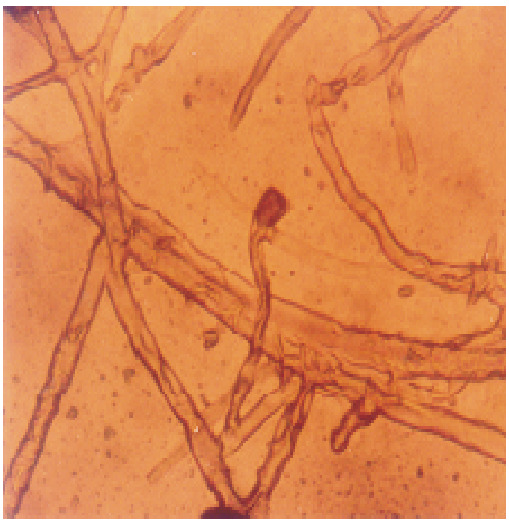

Figure 6. Pyriform collumelum (with truncate base) of *M. circinelloides* cultivated in GYPA; x2000 mag.

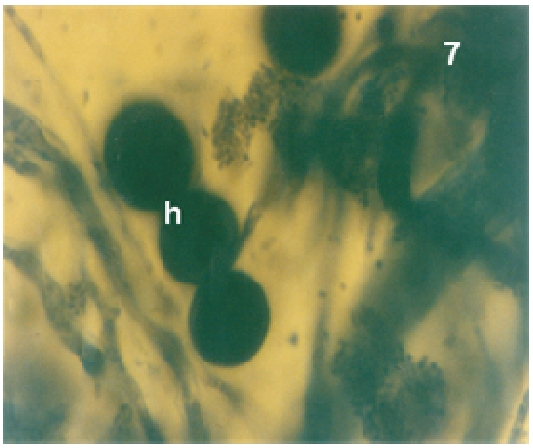

Figure 7. Holoblastic (h) conidia of *M. circinelloides* in K^+-enriched medium; x2000 mag (Omoifo, 2005).

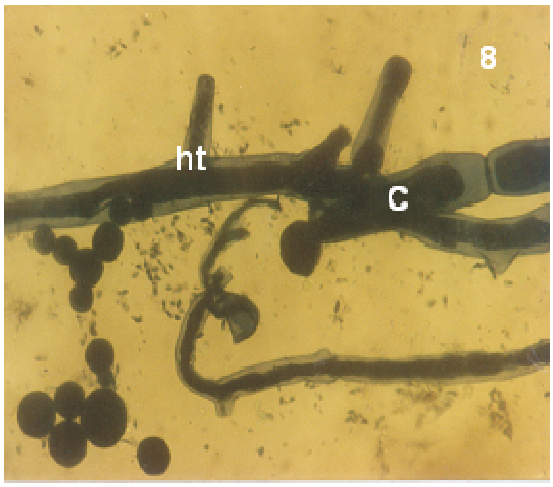

Figure 8. Holothallic growth (ht) with conidiophore (c) of *M. circinelloides* in K^+-enriched medium; x2000 mag (Omoifo, 2005).

were inoculated into a chemically defined medium with the carbon-substrate being the only preformed organic source. Although the headspace (atmosphere of the incubation) culture was not strictly controlled, buffering at pH 4.5 restricted reactions within the medium. A study showed that deviation from this initial value does not exceed 0.2 units throughout the growth transformation process (Omoifo, 2005). Spores convert to several anamorphs including holoblastic conidia (Figure 7) holothallic conidia (Figure 8), enterothallic conidia (Figures 7 and 9), thallic growth, with vesicular conidia headgroup (Figure 10), determinate septate thallic growth (Figure 11) and yeast cells (Figure 12).

MORPHOGENESIS

This is the sequence of interrelated events that goes to

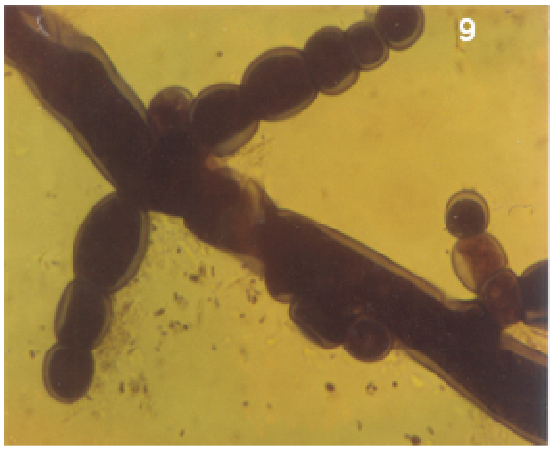

Figure 9. Enterothallic conidia (ec) of *M. circinelloides* in K^+-enriched medium; x2000 mag (Omoifo, 2005).

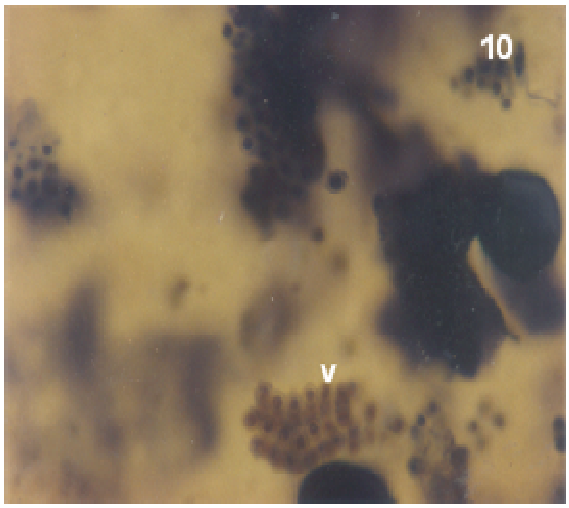

Figure 10. Vesicular conidia head group (v) of *M. circinelloides* in K^+-enriched medium; x2000 mag (Omoifo, 2005).

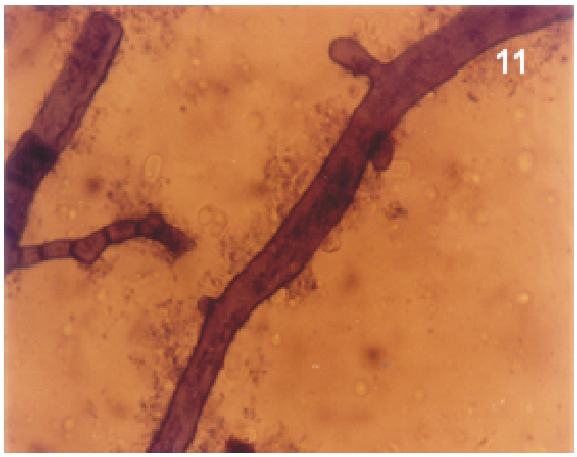

Figure 11. Septate thallic growth of *M. circinelloides* in K^+-enriched medium; x2000 mag (unpublished).

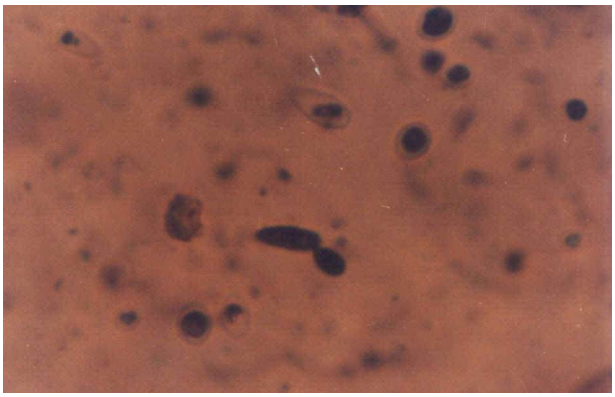

Figure 12. Yeast cells of *M. circinelloides* in K^+-enriched medium; x2000 mag (unpublished).

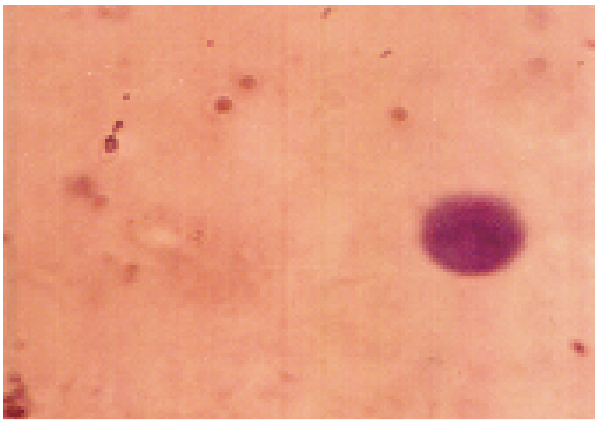

Figure 13. Growth sphere of *M. circinelloides* in myolnositol-enriched medium.

define the pattern and subsequent induction of a particular anamorph, including yeast cell, holoblastic conidia, holothallic conidia, and vesicular conidia headgroup. The focus here, is on the conversion of sporangiospores to the yeast morphology, which proliferated by terminal budding.

As the spore grows into a growth sphere (Bartnicki-Garcia and Lippman, 1987), there is an increase in size, *de novo* synthesis of cell wall from the inner layer of the sporangial wall and this increases the intensity of the growth sphere wall (Figure 13); also there is consequent increase of the surface area of the cell. Accompanying these changes in our synthetic broth is the inherent granulation of the cytoplasm. This is multiple nucleation as the numerous granular units can be directly observed as distinct individual units under the light microscope. This is in contrast to the 1-8 nuclei observed in a growth sphere by Bartnicki-Garcia and Nickerson (1962b). When growth sphere envelope undergoes lyses, nucleates are exposed in the medium (Figure 14). This is different from cell wall rupture whereby ghost cell or carcass is conspicuously left in the medium (Omoifo, 2005). Initially clustered, the individual units thereafter disperse by the apparent current prevailing within the medium (Figure 15). From non-determinate or coarse shape, the units acquire internal dimensions thus becoming shaped, globose or rod-shaped protoplasts (Figure 16). Although the specific sizes have not been determined, the relative sizes of protoplasts are sufficiently contrasting under the light microscope, x 2000 magnification. Figure 17 showed protoplasts with conspicuous internal dimensions, which have also acquired specific shapes: globose and rod-shaped. The progressive increase in sizes of the units is seen as they acquire cell walls and hence dubbed emergent yeast (Figure 18). There is further enlargement of size as the yeast matures, which subsequently becomes terminal budding (Figure 19).

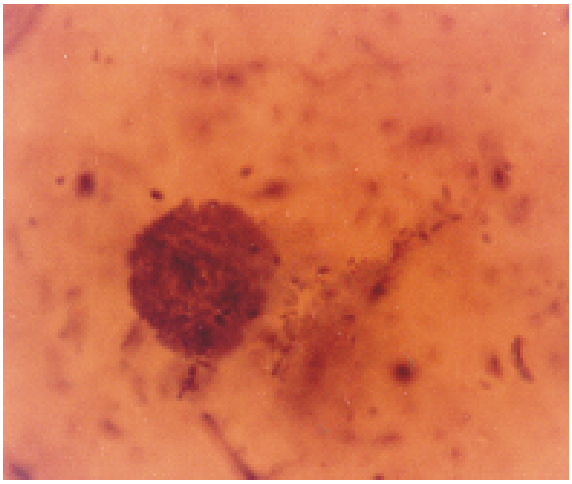

Figure 14. Growth sphere of *M. circinelloides* envelope compose from which the cell wall has lysed.

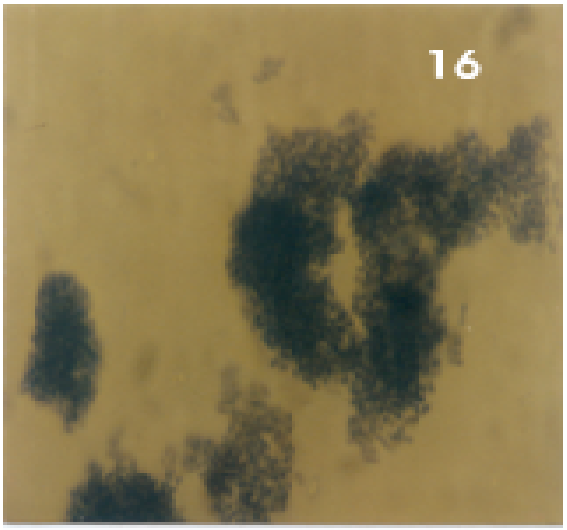

Figure 16. Protoplast initial of *M. circinelloides* in K^+-enriched medium; x2000mag (Omoifo, 2005).

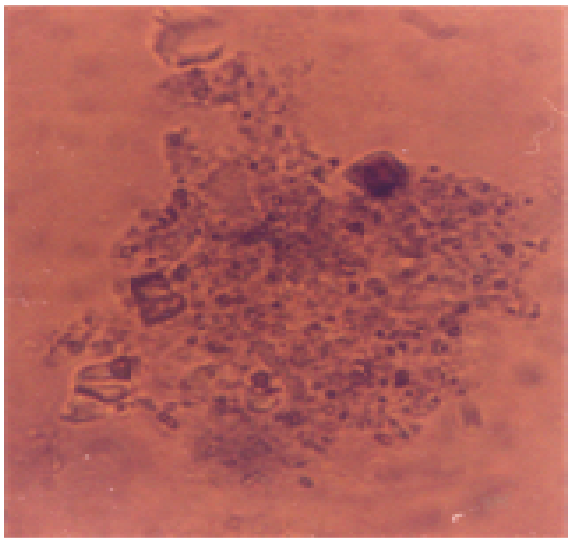

Figure 15. Dispersing cytosolic nucleates x2000 (unpublished).

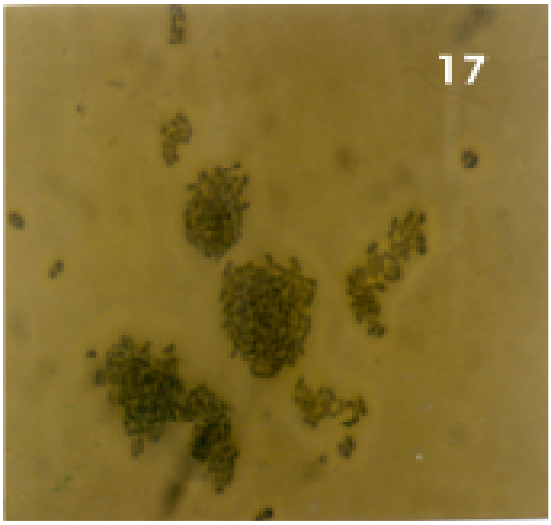

Figure 17. Globose and rod shaped protoplasts of *M. circinelloides* in K^+-enriched medium; x2000 mag. (Omoifo, 2005).

GROWTH PATTERN

Four growth phases were delineated for the tentative form-genus, *Dimorphomyces pleomorphis* strain C13, IMI W5132B (Omoifo, 1996). Cell envelope lysis hence cytoplasmic contents, tiny, follow isotropic growth, which occurred in phase 1, and coarse units, are exposed to the medium of growth. This is found in first part of phase 11 of growth of *D. pleomorphis* strain C13, IMI W5132B. Thereafter protoplasts are formed and subsequently assumed yeast form, which become predominant in phase 111 of peptone-incorporated broth but the only form in $(NH_4)_2SO_4$ broth. However, these phases were said to be pre-logarithmic (Omoifo, 1996).

If the period of isotropic growth and conversion of protoplast to the yeast form, which are all structural adjustments as phenotypic modifications, including inherent cellular differentiation and further metabolic adjustment to the new state of nutrition is taken into consideration, then the lag phase of *D. pleomorphis* strain C13, IMI W5132B took a whooping 72 h period (Figure 20). On the hand, *M. circinelloides* underwent these physiostructural modifications in a 42 h lag and thereafter assumed a sigmoid growth pattern at termination of the experiment (Omoifo, 2005); this, under the same set of conditions that gave a longer lag for *D. pleomorphis* strain C13, IMI

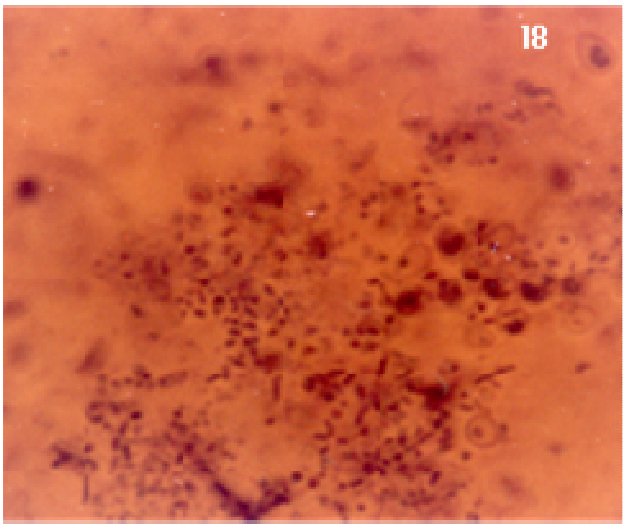

Figure 18. Emergent yeast cells of *M. circinelloides* in zinc and myoinositol enriched medium; x2000 (unpublished).

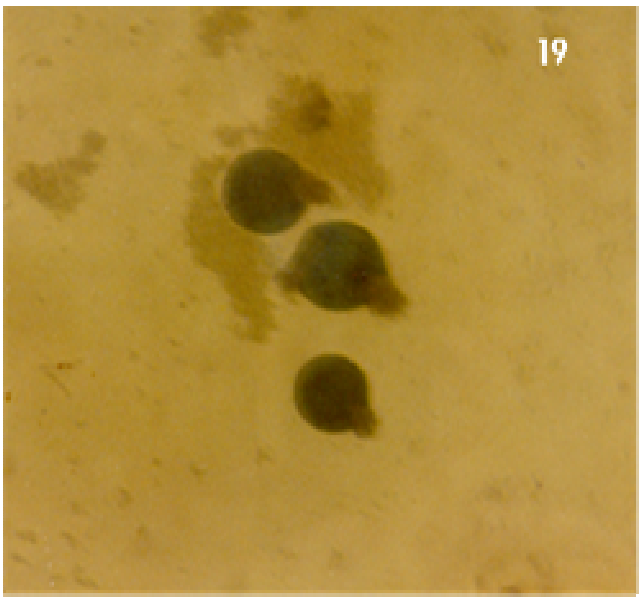

Figure 19. Terminal budding yeast cells of *M. circinelloides* in K^+-enriched medium; x2000 mag (Omoifo, 2005).

W5132B (Omoifo, 1996). Sig-moid growth pattern was further confirmed for *M. circinelloides* when the medium of growth was incorporated with different levels of myoinositol and zinc (Omoifo, 2006a).

Description of sigmoid pattern by *M. circinelloides* is in a cooperative system (Omoifo, 2005). Such conclusion was reached following incorporation of different levels of K^+ and Na^+ ions into the growth medium thereafter monitored through 120 h when sigmoid growth pattern resulted. This is shown in Figure 21. In a less cooperative system, a two-phase growth pattern occurred. It is significant to point out that beyond the transient forms, anamorphic expression in the *D. pleomorphis* strain C13, IMI W5132B cultivation was solely the yeast morphology. In contradistinction, thallic subtypes including holoblastic, holothallic, and enterothallic conidia, vesicular conidia headgroup in addition to the yeast form occurred during *M. circinelloides* cultivation and that the less cooperative the system was biophysically, the higher the preponderance of the subtypes, and hence farther from the sigmoid pattern of growth (Figure 22). A further study illustrating cooperation between the bulk medium and the organic content, i.e. the inoculum, resulting in specific phenotypic expression showed several growth patterns of this *M. circinelliodes* including 1-phase, 2-phase and sigmoid growth phases (Figure 23).

Conclusion

In synthetic submerge, sporogenesis typical of the Zygomycetes does not occur, since it requires formation of aerial hyphae and sporangia. Instead vesicle formation from which radiate numerous chains of conidia occur. Apical growth, a characteristic of hyphal growth occurred but in this case (broth), mycelium is septate and determinate and does not end in collumelae. In some thallic growth form, conidiophore is produced and thereafter, conidia. Also observed were holoblastic conidia with incomplete formation of septum (Omoifo, 2005; Omoifo and Omamor, 2005).

Dimorphism in *M. circinelloides* goes beyond the ability of sporangiospores to grow in the distinct isotropic and apical forms: growth sphere/yeast and mould. Here is a demonstration of development of specific intermediate forms during the transformation process: nucleates and protoplasts. But importantly, there is no change in total genetic information since the final product of each of the forms is always a return to the starting state: sporangiospore (Omoifo, 2003).

This chronicle has shown that terminal budding yeast cells, a characteristic of the higher fungi, can also be derived from the Zygomycetes in modified environments in a predictable manner. It also means that it can be approached with similar analytical procedures, including biophysical, biochemical and molecular techniques as is done for instance, in *Saccharomyces cerevisae.* Additionally, and beyond this morphology is the examination of its primordial source: the phenomena of cytosolic nucleation and protoplast formation.

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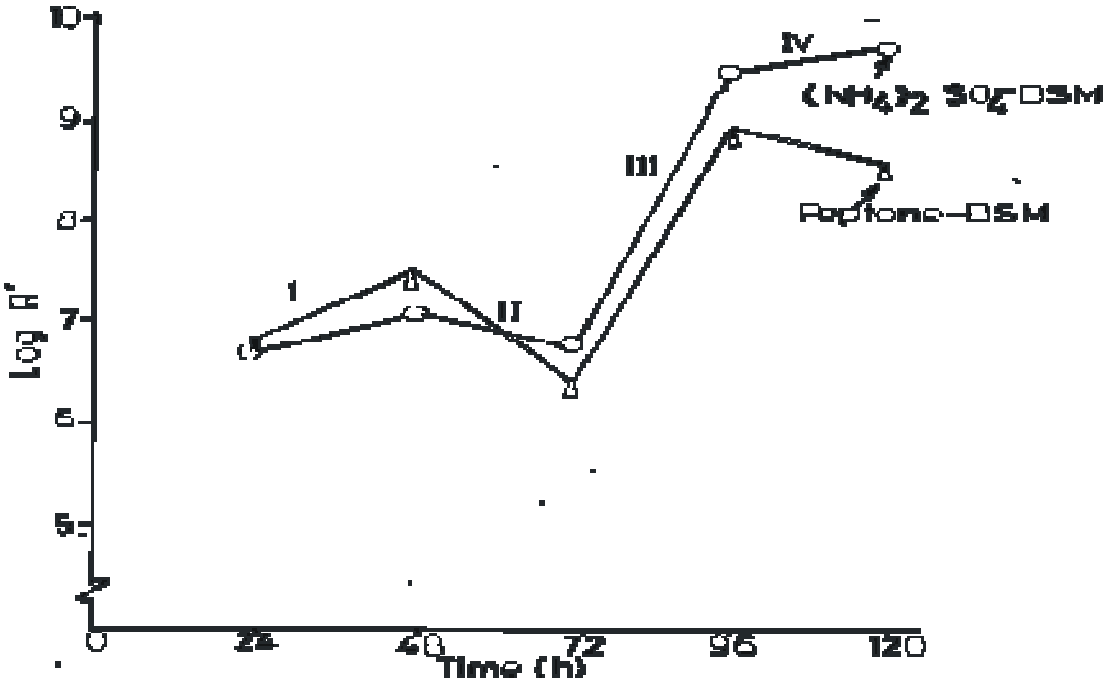

Figure 20. Growth of *D. pleomorphis* prior to exponential growth when cultivated in buffered basal salts media pH 4.5, temp. 20°C; I = isotropic growth, II = lysis and release of protoplasmic contents, III = phenotypic adjustment, IV = metabolic adjustment (modified from Omoifo, 1996).

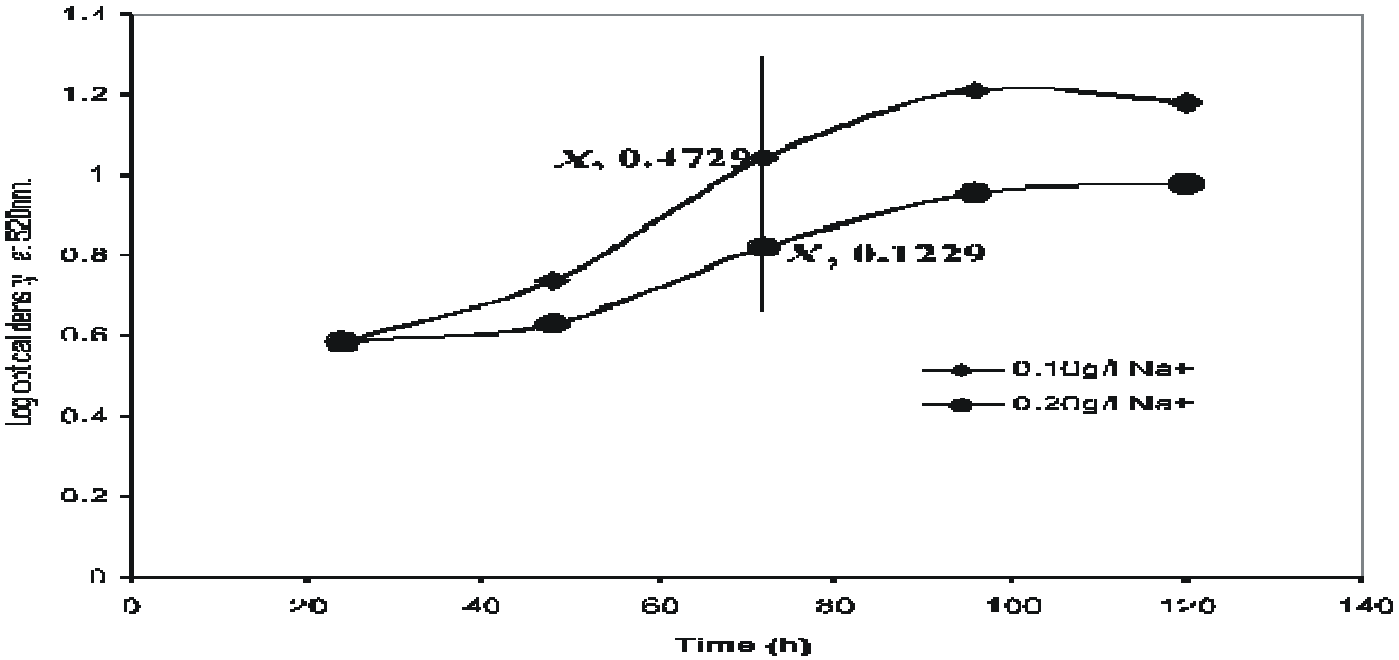

Figure 21. Sigmoid curves and Na+ influx rates at exponential growth phases of *M. circinelloides* Tieghe cultivated in broth charged with 1.0 g/l k+. x, Na + influx rate (modified from Omoifo, 2005).

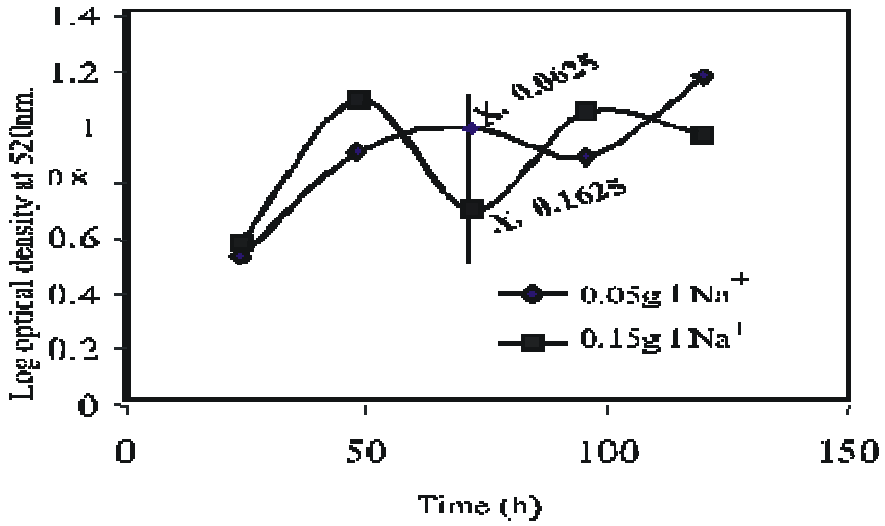

Figure 22. Non cooperative growth curves for sporangiospore-yeast transformation of, and Na+ influx rates at exponential growth phases of *M. circinelloides* Tieghe cultivated in broth charged with 1.0 g/l k+. x, Na + influx rate (modified from Omoifo, 2005).

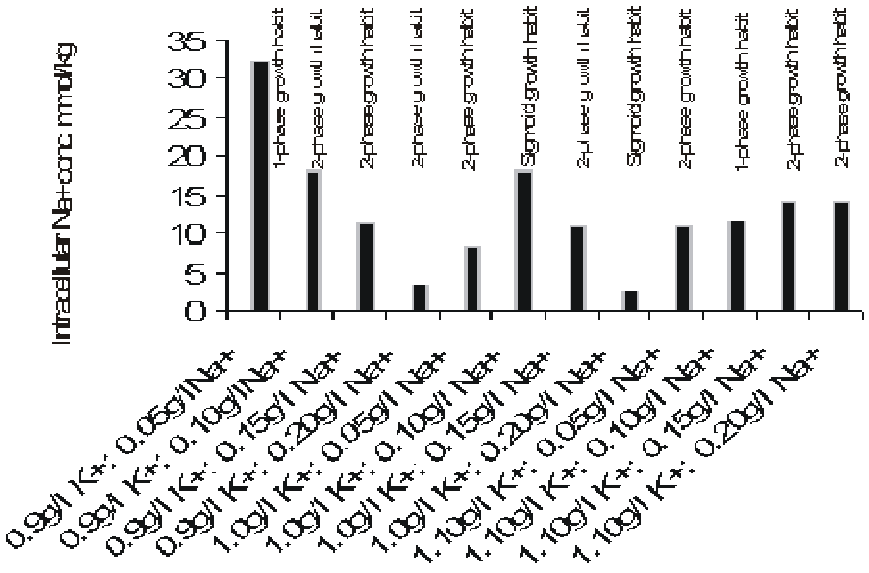

Figure 23. Histograms showing Na+ accumulations after 72 h of growth and growth patterns exhibited by *M. circinelloides* in synthetic broth (unpublished).

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