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Full Length Research Paper

Sporangiospore-yeast transformation of *Mucor circinelloides*: Ionic circulation, chemical potential, sodium influx rate and morphogenesis

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Measurement of intracellular ion concentration during sporangiospores-yeast transformation of *Mucor circinelloides* Tieghem in K^+- mediated (0.90 to 1.10 g/l) and Na^+-modulated (0.05 to 0.20 g/l) multiionic broths, pH 4.5, temperature 20°C, showed that (a), transmembrane ion flux was continuous during the growth period (b), Na^+ efflux varied concurrently with K^+ influx and vice versa (c), induced morphologies varied: growth spheres, enterothallic-, holothallic-, holoblastic- conidia, septate hyphae with vesicular conidial head groups, and terminal budding yeast cells but protoplasts and granular particles were transient (d), time-course plots of log transformed optical density measurements gave sigmoid growth pattern principally at 1.0 g/l K^+ and 0.10 g/l Na^+ incorporation where the induced morphology was mainly terminal budding yeast but primary and secondary growth optima occurred at the other levels and these were thought to result from determinate thallic growth: septate hyphae transformed into enterothallic conidia; cytosolic nucleates/granular particles could be extruded after thallic cell wall lyses or conidial burst, which then collapsed; the granular units thereafter became protoplasts and could assume the yeast morphology (e), ionic profiles showed correlation with growth phases: lag phase was accompanied with Na^+ efflux with simultaneous influx of K^+; exponential growth phase- rapid influx of Na^+ and concurrent efflux of K^+; stationary phase- rapid efflux of Na^+ with simultaneous, albeit slow intracellular accumulation of K^+ . It was strongly suggested that ion fluxes were mediated by the P-type, plasma-membrane localized, cation ATPases. This study shows that morphological expression was correlated with the intracellular ion contents. The chemical potentials (*m*) were calculated at the pre- logarithmic growth phases and these influenced cytosolic nucleation. As the magnitude of *m* increased, cytosolic nucleates were less readily formed. Apparently, a balance of Na^+ and K^+, deputed as critical concentration (*cc*), was required for cytosolic nucleation. But transition from protoplasts to the yeast form was thought to be dependent on the magnitude of the Na^+ influx rate (x). We conclude that highly functional cooperativity existed between *m*, *x*, as well as *cc*, for successful transformation of sporangiospores to terminal budding yeast cells.

Key words: Cytosolic nucleation, ionic flux, critical concentration, chemical potential.

INTRODUCTION

In the study with *Mucor circinelloides* Tieghem, observations showed that the olive-green septate hyphae with vesicular conidial chains were a recurrent feature when the K^+ concentration was varied in the buffered broths (preceding reports). It was also shown that protoplasts were a dominant feature in all K^+-incorporated media. Since it was not induced in the absence of K^+, it was therefore concluded that K^+ was of absolute necessity for the formation of protoplasts, which preceded the yeast morphology. The study also showed that mature yeast cells, though scanty were induced at low K^+ concentration, but they were numerous at high K^+ level.

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Buffering the medium of growth created H^+ concentration differential between the inner and outer media. This gave rise to transmembrane- pH- gradient with its inherent potential energy. Consequently, primary active ion transport led to the buildup of H^+ in the bulk medium. In the K^+-mediated media, yeast cells became more robust and, then preponderant as the K^+ concen-tration increased. The yeast cells became predominant at 1.00 g/l K^+ where the intensity of proton release from the intracellular medium had the highest value (preceding reports).

Thus, the electrolytes, H^+ and K^+, had great impact on the formation of protoplasts and the development of the yeast morphology thereof. Major cell biology texts, including those by Conn and Stumpf (1976), Berns (1977), Dawes (1986), Zubay et al. (1975), Abeles et al. (1992), Albert et al. (1994) and Delvin et al. (1997) have explained that K^+, which is intimately associated with Na^+ movement play essential role in critical physiological functions, as they are capable of generating action potentials and ion currents through the intracellular membrane.

Since K^+ has inherent antiport-electrogenic relationship with Na^+, perhaps this would influence physiological activities in the transformation processes leading to change in morphological expression. This report is on the effect of intracellular K^+ and Na^+ on the transformation of sporangiospores of *M. circinelloides* Tieghem. It also correlates morphological expression with intracellular ion content.

MATERIALS AND METHODS

Organism and inoculum preparation

Procedure for obtaining the strain of *M. circinelloides* has been described. It was originally isolated from decayed fruit of soursop, *Annona muricata* L., obtained from the floor bed of the soursop tree and purified on glucose-yeast extract-peptone agar (GYPA: 10-3-5-20 g/l), while inoculum was prepared as previously stated (preceding report) by pouring sterile distilled water over aerobically grown cultures and then washing the spore suspension in 3 changes of sterile distilled water and thereafter making it up 10^6 spores/ml.

Chemicals and media preparation

The procedure used in preparing glucose- substrate multiionic broth as reported by Omoifo (1996b) was followed. Only 3 levels of K^+ (0.90, 1.00 and 1.10 g/l) were used: based on the yeast-inducing capabilities, as determined in the preceding studies. Four concentrations of Na^+ (0.05, 0.10, 0.15, 0.20 g/l) were combined with levels of K^+. Duplicate experiments were set up for each test.

Inoculation and culturing conditions

These were previously reported (1996a, b). Incubation temperature (20°C), ambient was used for growth studies.

Harvesting, microscopic examination, optical density determination and photomicrography

At intervals of 24 h, 10 ml of the culture was removed with pre-sterilized pipette, one for each culture medium, into pre-labeled boiling tubes. The flasks were vigorously shaken to rake up the sediments before withdrawing the fluid in aseptic conditions; they were thereafter, returned for further incubation.

Optical density was determined as previously stated in the preceding report. Similarly followed were procedures for microscopic examination and photomicrography. Staining of preparations was with lactophenol-in-cotton blue. Micrographs were obtained with a Leitz Wetzlar Ortholus microscope (Germany) attached with an Ernst Leitz camera. Morphological description was referenced with Alexopoulos and Mims (1979), Kendrick (1971) and Talbot (1971).

Cellular concentration of cations

For the determination of intracellular ion content, the method followed was that of Camacho et al. (1981) with modifications. The culture suspensions were centrifuged at 5000 rpm for 10 min at 25°C (MSE 18). The supernatant were each decanted and 5 ml of 20 mM $MgCl_2$ solution were added and centrifuged for 10 min, in a very rapid operation. A 5 ml of 0.2 M HCl and 5 ml of 10 mM $MgCl_2$ were added to re-suspend the cells. These were then poured into factory-made sterile plastic bottles and thereafter left for ion extraction for 24 h. The extract was centrifuge for 15 min and the supernatant was obtained for cation determination using Digital Flame Analyzer (ref FGA –350-L; Gallenkamp, England).

Analysis of data

This was done in Microsoft Excel. Growth data were log transformed.

RESULTS

Effect of K^+ and Na^+ incorporation on intracellular concentration of K^+ and Na^+ in *M. circinelloides* Tieghem

During the cultivation of *M. circinelloides* Tieghem in the media incorporated with varying concentrations of K^+ and Na^+, the electrolytes were continuously present in the intracellular medium. However, the concentrations varied. In the 0.05 g/l Na^+-modulated medium, there was rapid depletion of Na^+ from the intracellular medium and this came to a minimum after 48 h of growth. It increased rapidly, and subsequently fell, after 72 h. The diminishing trend was again reversed after 96 h of growth. In the case of intracellular content of K^+, a decrease also occurred but it was half the magnitude of intracellular Na^+ content. Still, the pattern of decrease and increase was similar to that of Na^+ (Figure 1a)

The pattern observed for the intracellular concentration of Na^+ in the 0.05 g/l Na^+- modulated broth was largely true for the other levels (Figure 1a to c), except in the 0.20 g/l Na^+ broth (Figure 1d) where a steady decline

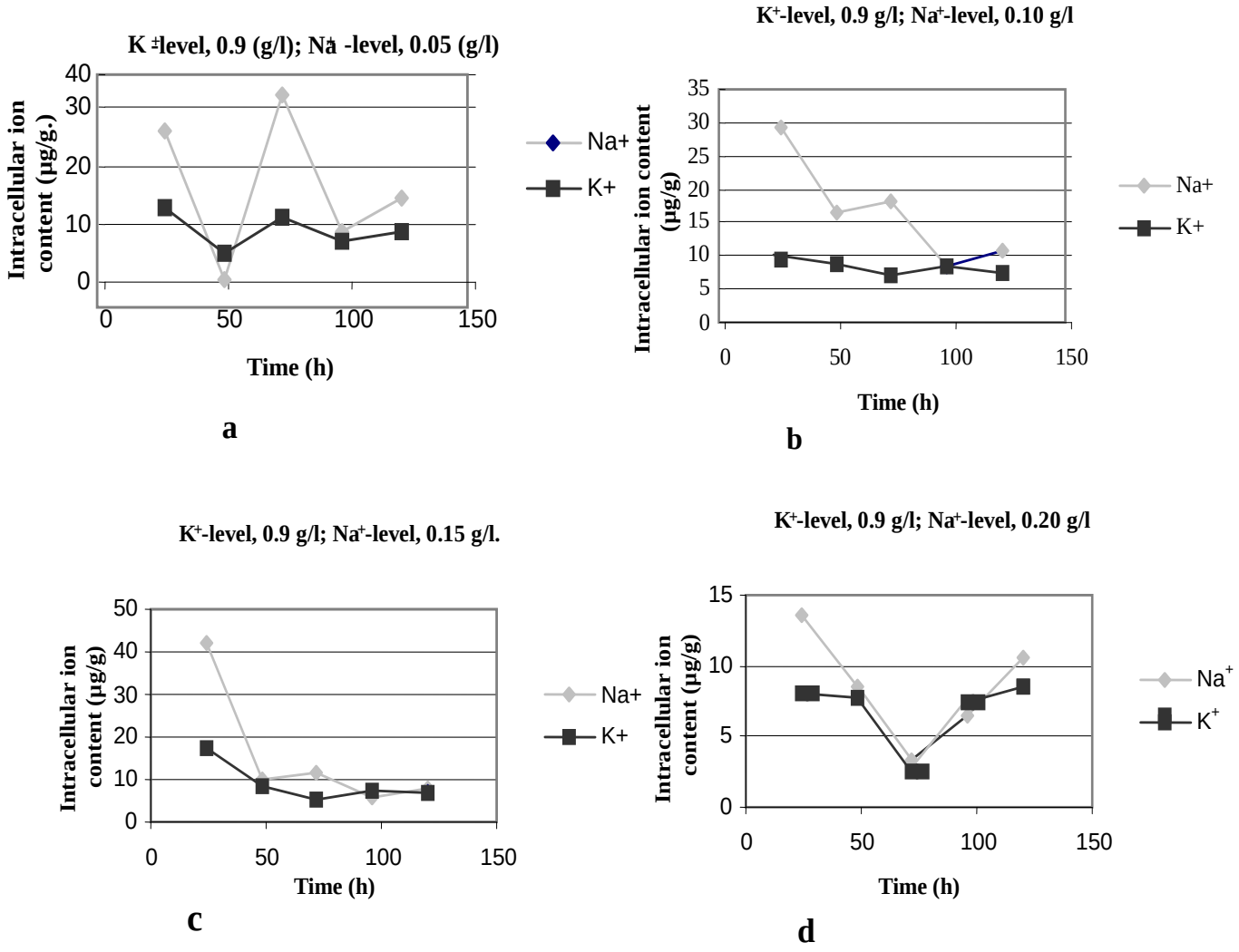

Figure 1. Intercellular ion variation (K^+, 0.90 g/l; Na^+, 0.05 to 0.20 g/l) during sporangiospore-to-yeast transformation of *M. circinelloides* Tieghem cultivated in glucose-substrate multiionic broth for 120 h at pH 4.5, temperature of 20°C (ambient).

occurred till 72 h after inoculation. Thereafter, incremental measurement revealed a trend in the opposite direction. In this medium, internal K^+ content appeared to stabilize within the first 48 h of growth. It then fell to a minimum at 72 h where it also inflected; the trend line was in the opposite direction. The rapid accumulation of internal K^+ diminished after 96 h of growth of the organism. The decrease in internal K^+, up to the point of inflexion at 72 h and its subsequent increase, appeared more benign at the 0.10 and 0.15 g/l Na^+ variations.

Intracellular ion variation also occurred at the 1.0 g/l K^+-mediated 0.05 to 0.20 g/l Na^+-modulated broths (Figure 2a to d). The pattern description for intracellular ion content given earlier was most cryptic at 0.10 g/l Na^+-modulated, 1.0 g/l K^+- mediated medium (Figure 2b). The profile at 0.20 g/l Na^+-modulated broth approximated this pattern (Figure 2d) but others showed wide deviations from it (Figure 2a and c). If the regularity of intracellular ion profile in the 0.1 g/l Na^+, 1.0 g/l K^+ culture (Figure 2b) was assumed as the standard for a specific physiology, then deviated forms differed from such.

When the K^+ level was increased to 1.10 g/l, the combination (Figure 3a to d) of K^+ and Na^+ intracellular content did not reflect the profiles shown in Figure 3b, even though Na^+ profile alone approximated the description (Figure 3a to c).

Effect of K^+ and Na^+ incorporation on morphology of *M. circinelloides* Tieghem

The 0.9 g/l K^+- mediated and 0.05 to 0.20 g/l Na^+-modulated broths

Two types of septate mycelia were induced in the 0.9

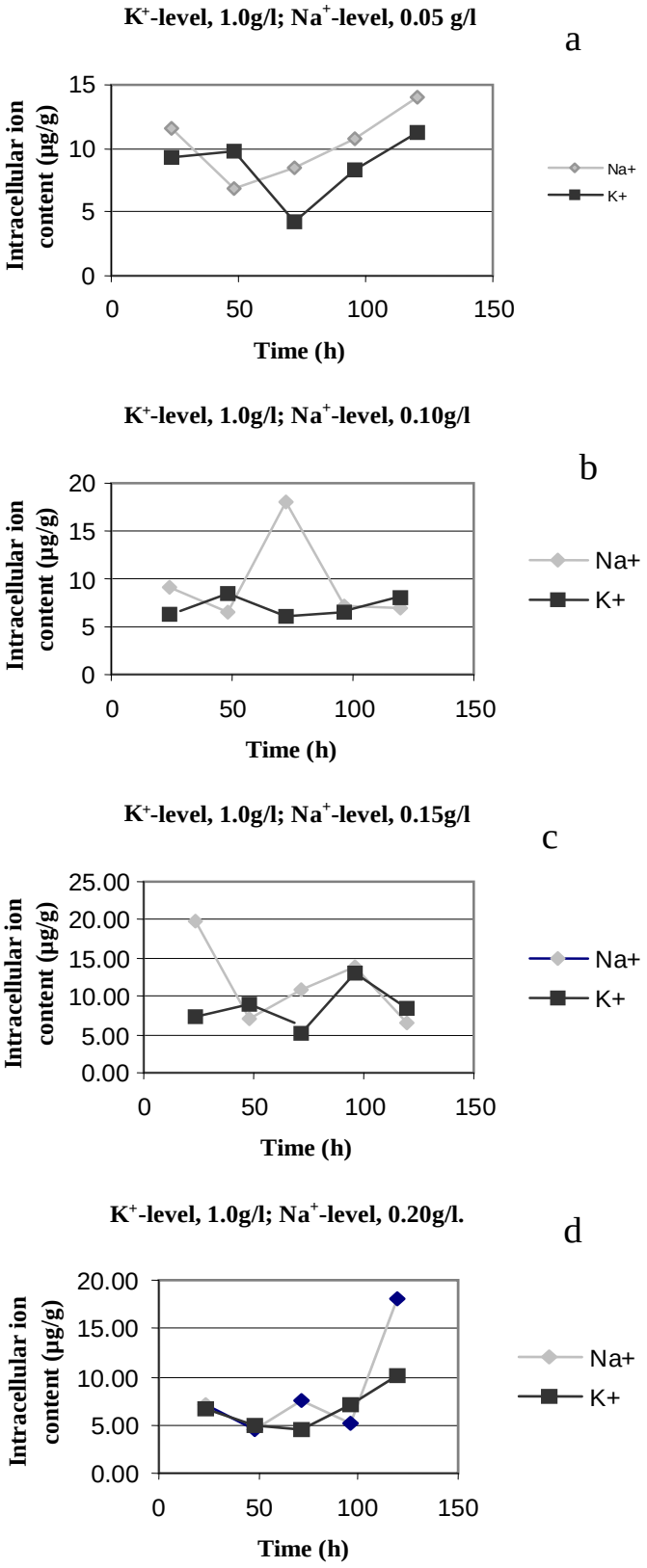

Figure 2. Intracellular ion variation (K^+, 1.0 g/l; Na^+, 0.05 to 0.20 g/l) during sporangiospore-yeast transformation of *M. circinelloides* Tieghem cultivated in glucose-substrate multiionic broth for 120 h at pH 4.5, temperature 20°C (ambient).

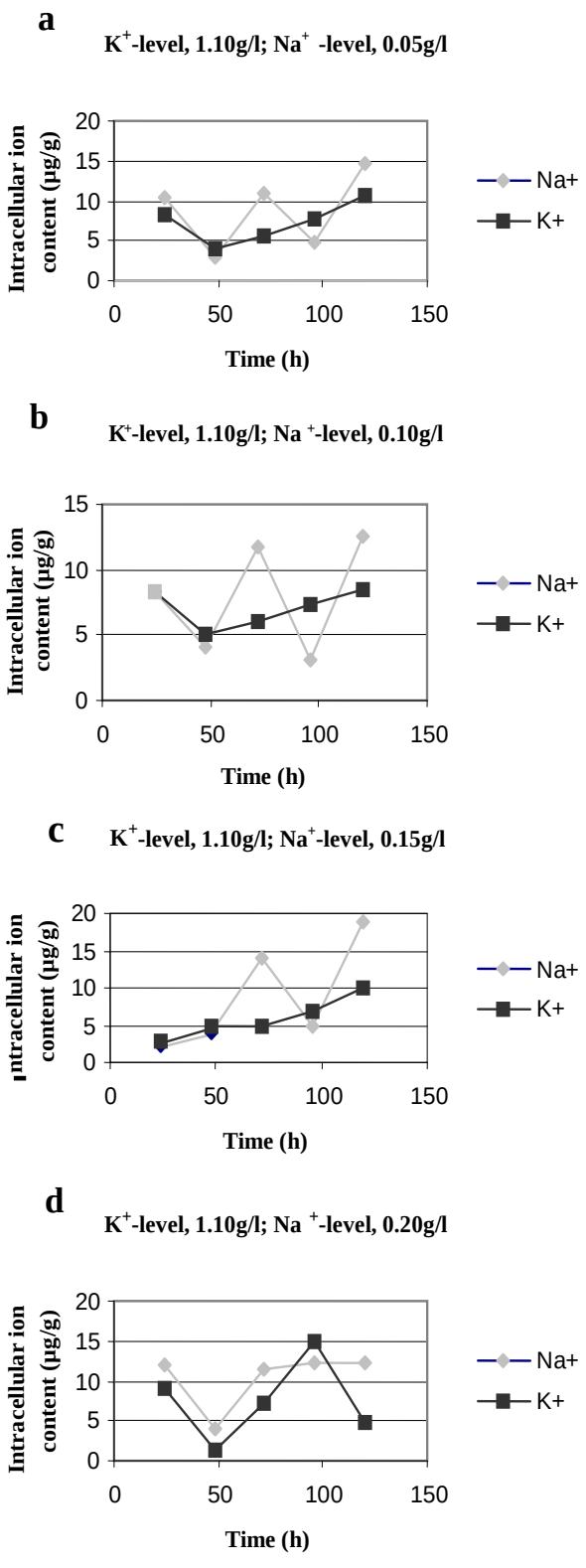

Figure 3. Intracellular ion variation (K^+, 1.10 g/l; Na^+, 0.05 to 0.20 g/l) during sporangiospore-yeast transformation of *M. circinelloides* Tieghem cultivated in glucose-substrate multiionic broth for 120 h at pH 4.5, temperature 20°C (ambient).

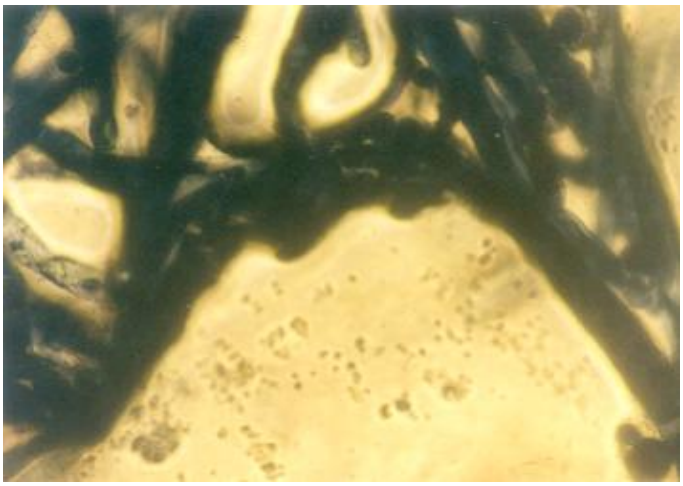

Figure 4. Stained septate hyphae (out of focus) and unstained granular particles of *M. circinelloides* Tieghem induced in 0.90 g/l K^+- mediated and 0.05 g/l Na^+- modulated multiionic broth at 2000x magnification.

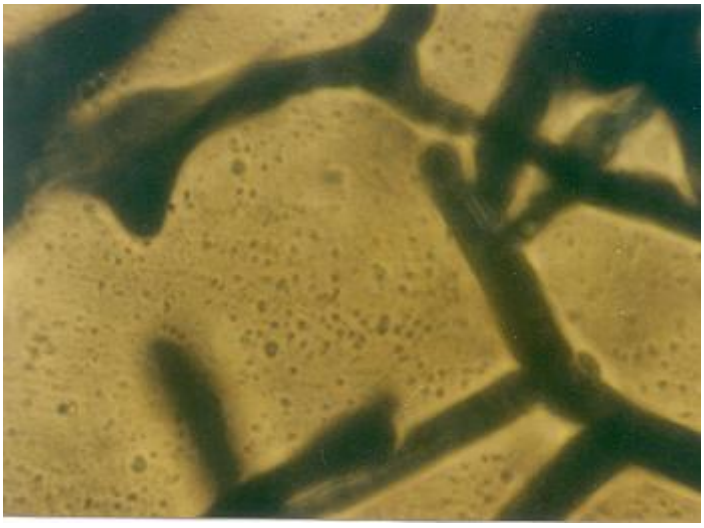

Figure 5. Stained septate hyphae (out of focus) and intermediately stained granular particles of *M. circinelloides* Tieghem from a section of Figure 5 at 2000x magnification.

g/l K^+- mediated and 0.5 g/l Na^+-modulated broth. These included thin walled, olive-green notched septate hyphae with conidiophores which terminated in enlarged subglobose vesicle from which radiated chains of globose to subglobose conidia (preceding reports) and double walled, heavily stained and highly branched septate hyphae forming a meshwork of mycelium (Figure 4), but could also give rise to thallic conidia. Granular units, unstained and orange, were also preponderant (Figure 4). As the granular units became stained as intermediates, that is, between unstained and the dark blue stain, the units approached a regular shape and subsequently assumed internal dimensions (Figure 5), hence becoming protoplasts, which were ovoid, short rod and bacterial-like in morphology. Shown in Figure 6 are clusters of the stained bacteria- like entities at x1000 magnification.

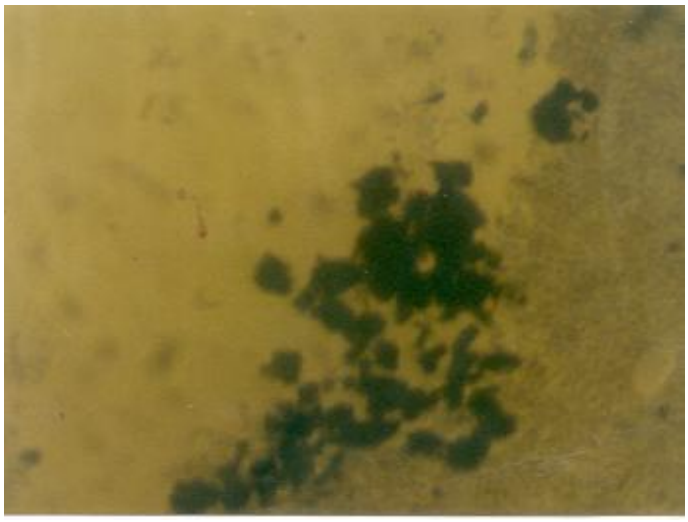

Figure 6. Clusters of stained protoplasts of *M. circinelloides* Tieghem induced in 0.90 g/l K^+-mediated and 0.05 g/l Na^+-modulated multiionic broth at 2000x magnification.

When this K^+ concentration was modulated with Na^+ at 0.10 g/l, similarly induced were (a) olive green septate hyphae with vesicular conidial head groups; (b) highly stained and branched hyphae with enterothallic conidia which could be given off in long or short chains: these filaments did not form a meshwork as in the lower Na^+ concentration; (c) holoblastic conidia; (d) conspicuous protoplasts; and (e) yeast cells, which predominated in the medium. Figures 7 to 9 showed terminal budding yeast cells induced in this medium with increasing magnification.

Modulation of 0.90 g/l K^+ broth with 0.15 or 0.20 g/l Na^+ also induced the aforementioned morphologies but variation in each type of morphology was greater. For instance, the protoplast became more bacteria-like, assuming several shapes: cylindrical, ovoid, short rods and in single or double, and enterothallic conidia which were oblong, rectangular or subglobose. Germlines and holothallic conidia were also present. Figure 10 shows thallic growth with clinging protoplasts at x1000 in the 0.90 g/l K^+- mediated and 0.15 g/l Na^+- modulated medium.

The 1.0g/l K^+- mediated and 0.05 to 0.20g/l Na^+-modulated broths

Modulation of the 1.0 g/l K^+- mediated broth with 0.05 g/l Na^+ induced several coexisting morphologies. Prominent

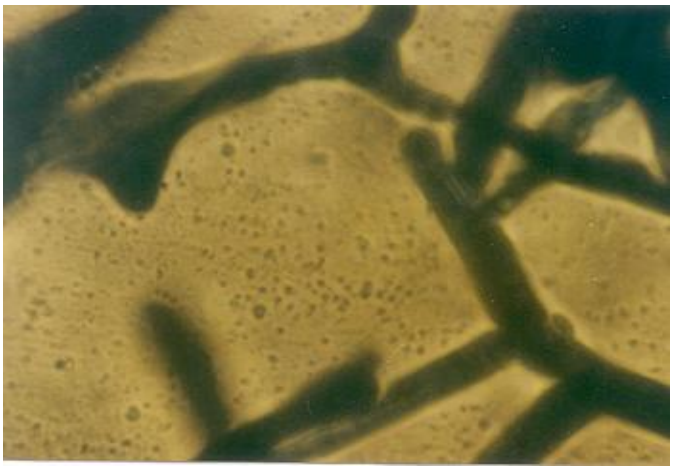

Figure 7. Septate hyphae, holoblastic conidia and yeast cells of *M. circinelloides* Tieghem induced in 0.90 g/l K^+- mediated and 0.10 g/l Na^+- modulated multiionic broth at 400x magnification.

Figure 10. Hyphae and adhering protoplasts of *M. circinelloides* Tieghem induced in 0.90 g/l K^+-mediated and 0.15 g/l Na^+- modulated multiionic broth at 1000x magnification.

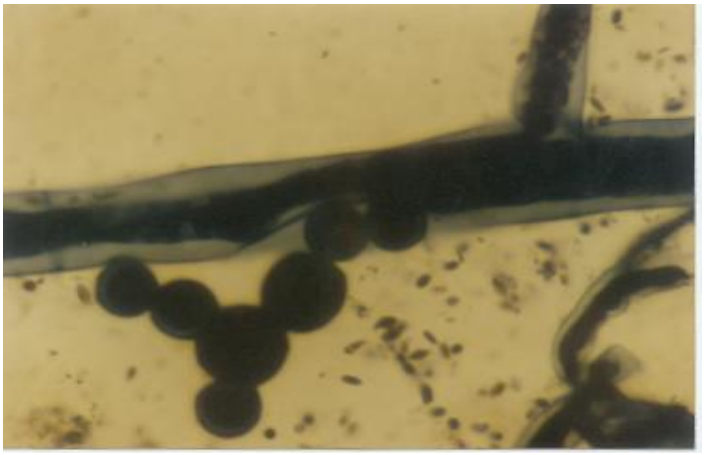

Figure 8. Yeast cells, holoblastic conidia and hypha at 1000x magnification.

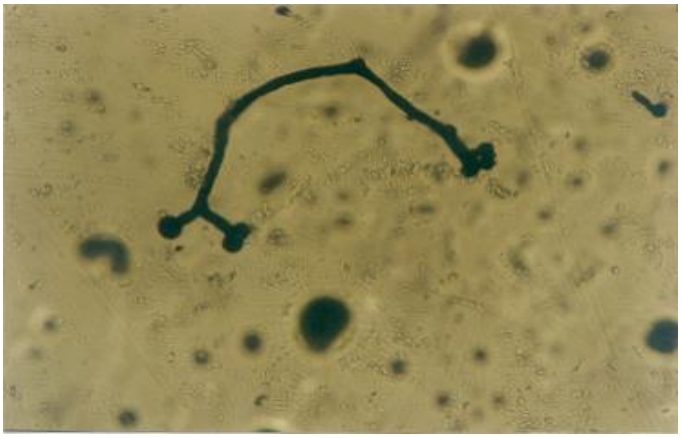

Figure 11. Determinate thallic growth and enterothallic conidia of *M. circinelloides* Tieghem induced in 1.00 g/l K^+- mediated and 0.05 g/l Na^+- modulated multiionic broth at 800 x magnification.

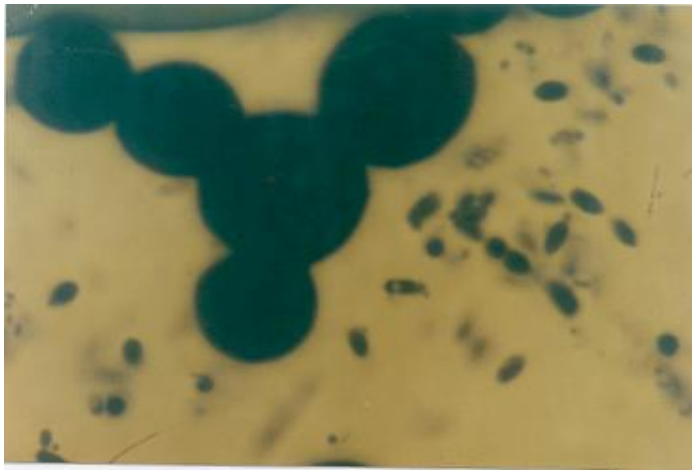

Figure 9. Yeast cells, holoblastic conidia and section of hypha in figure 8 at 2000x magnification.

Figure 12. Branched septate hypha and stained granular particles of *M. circinelloides* Tieghem induced in 1.00 g/l K^+- mediated and 0.05 g/l Na^+- modulated multiionic broth at 800x magnification.

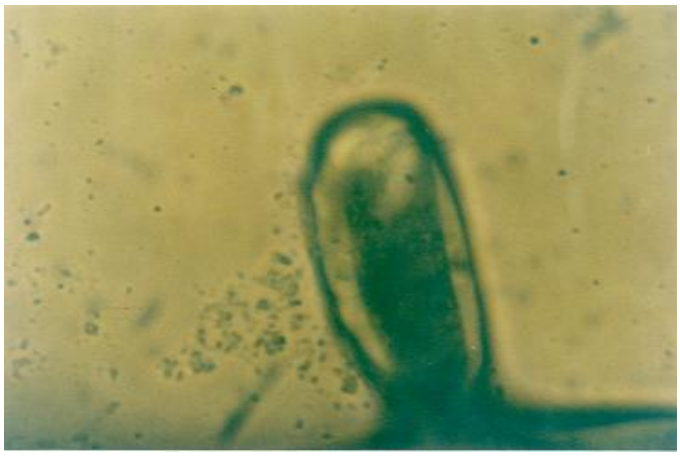

Figure 13. Stained granular particles, double cell wall of thallus and plasma membrane at 2000x magnification.

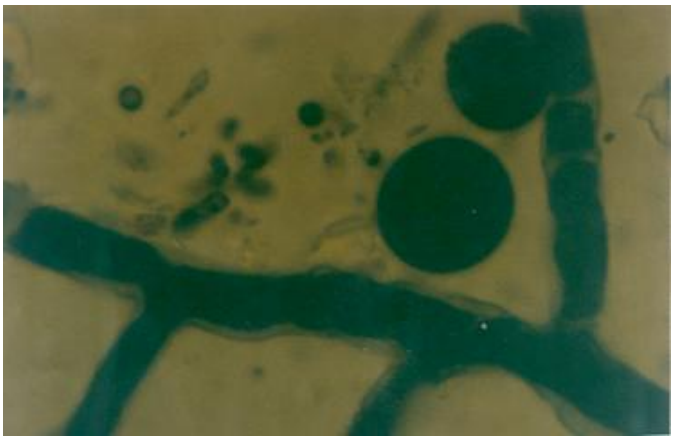

Figure 16. A section of Figure 15 with emphasis on the yeast cells, which were globose, oblong, obpyriform and terminal budding at 2000x magnification.

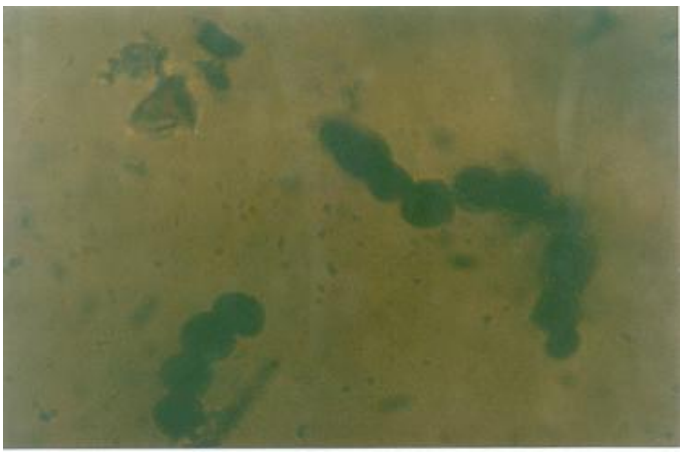

Figure 14. Holoblastic conidia of *M. circinelloides* Tieghem induced in 1.00 g/l K^+- mediated and 0.05 g/l Na^+- modulated multiionic broth at 800 x magnification.

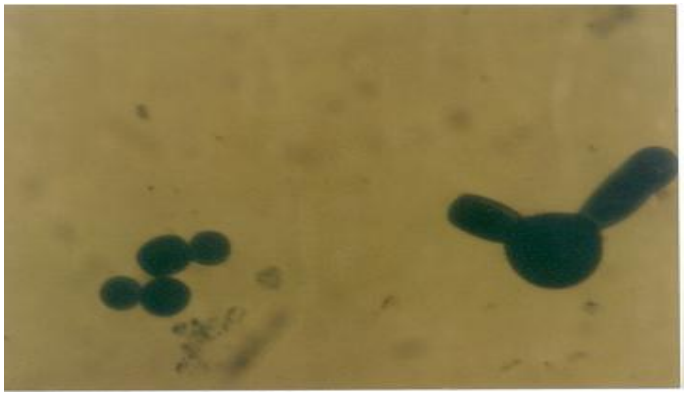

Figure 17. Germline, with double germ tube, holoblastic conidia and granular units of *M. circinelloides* Tieghem induced in 1.0 g.l K^+- mediated and 0.10 g/l Na^+- modulated multiionic broth at 800 x magnification.

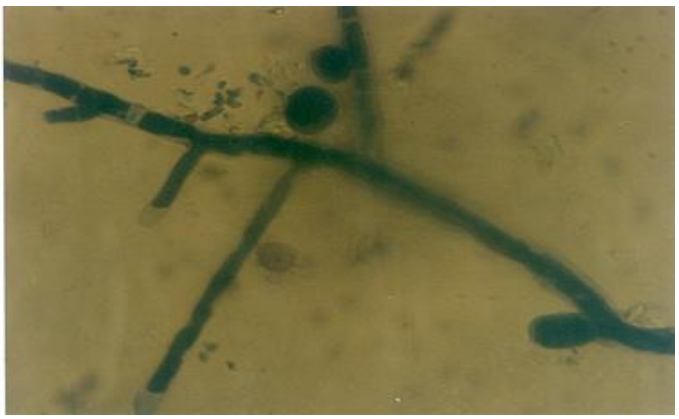

Figure 15. Growth spheres of branched septate hyphae and terminal budding yeast cells of *M. circinelloides* Tieghem induced in 1.00 g/l K^+- mediated and 0.05 g/l Na^+- modulated multiionic broth at 800x magnification.

was the olive green thin walled septate hyphae with vesicular conidial head groups, but these became atrophied. Determinate thallic growth, which could be severally branched, formed enterothallic conidia (Figure 11). Thalli were double walled and septate (Figure 12). Figure 13 is a higher magnification of Figure 12, showing the coexistence of branched thallus with enlarging particles in the same field range. The occurrence of thallic growth in this medium was however scanty. Holoblastic conidia were atrophied (Figure 14). Figures 15 to 16 show the coexistence of septate filaments, growth spheres and yeast cells obtained in the same focal field.

When modulation was with 0.10 g/l Na^+, still the olive-green septate hyphae with shortened conidiophores bearing vesicular conidial head groups were more atrophied. Also induced were holoblastic conidia and germlines with several germ tubes (Figure 17). Such germ tubes could originate from the same locus and, or

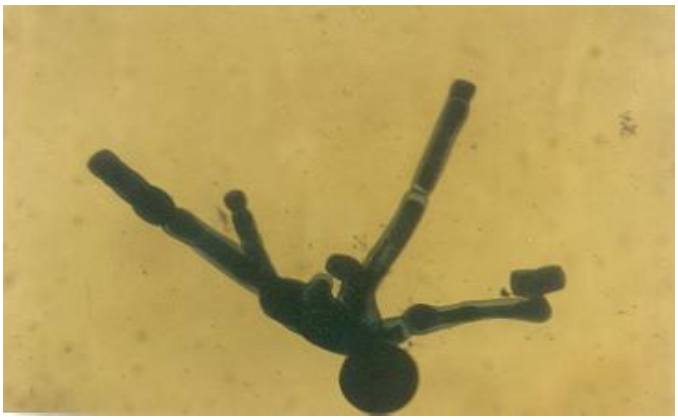

Figure 18. Growth spore, with single locus multiple germ tube of *M. circinelloides* Tieghem induced in 1.0 g.l K^+- mediated and 0.10 g/l Na^+- modulated multiionic broth at 800x magnification.

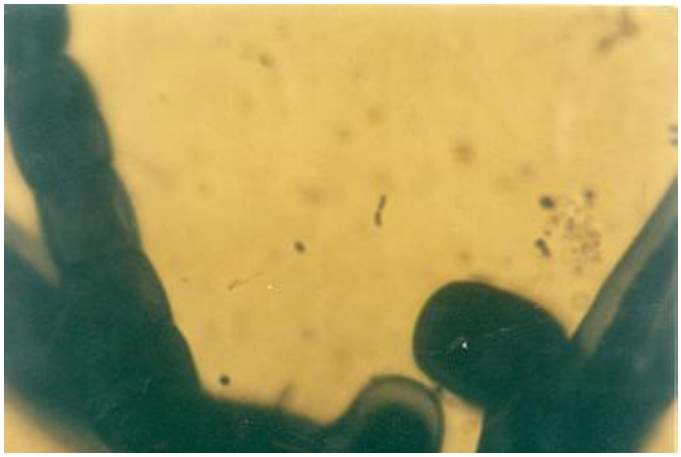

Figure 19. A higher magnification (2000x) of a section of Figure 18 showing the transforming protoplasts which are bacteria-like.

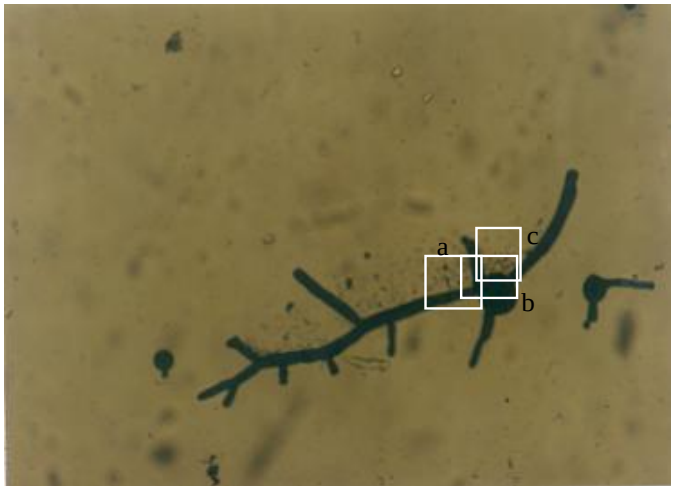

Figure 20. Germlines and branched thallic growth of *M. circinelloides* Tieghem induced in 1.0 g.l K^+- mediated and 0.10g/l Na^+- modulated multiionic broth at 200x magnification.

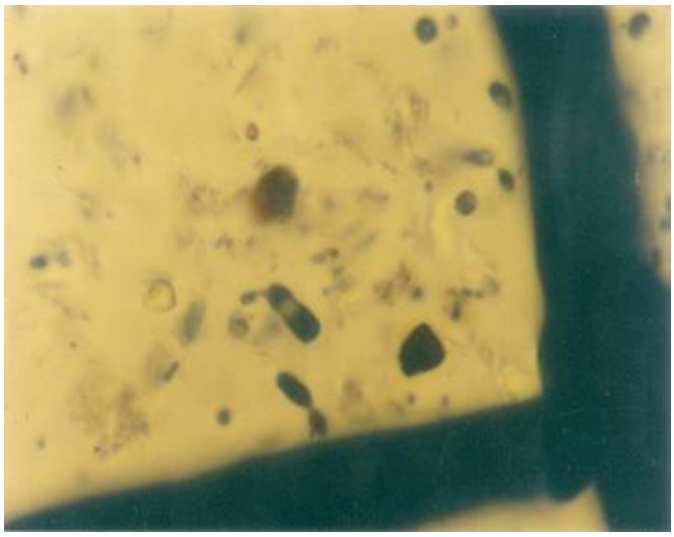

Figure 21. Different elevation of Figure 20, a, showing terminal budding yeast cells at 2000x magnification.

Figure 22. Another section of Figure 20, ***b,*** showing yeast cells at 2000x magnification.

became septate (Figure 18). Figure 19 is a section of Figure 18, which had transformed to enterothallic conidia; also observed was the bacteria-like cells (transforming protoplasts), in this micrograph. They occurred singly or in doubles. Branched thallic growth occurred but might not form mycelium (Figure 20); these were however few. Figures 21 to 23 show several aspects of the growth sphere in Figure 20, emphasizing the presence of terminal budding yeast cells, which could be globose, subglobose, pyriform, obpyriform or cylindrical. The yeast cell constituted the dominant morphology in this medium (Figure 24).

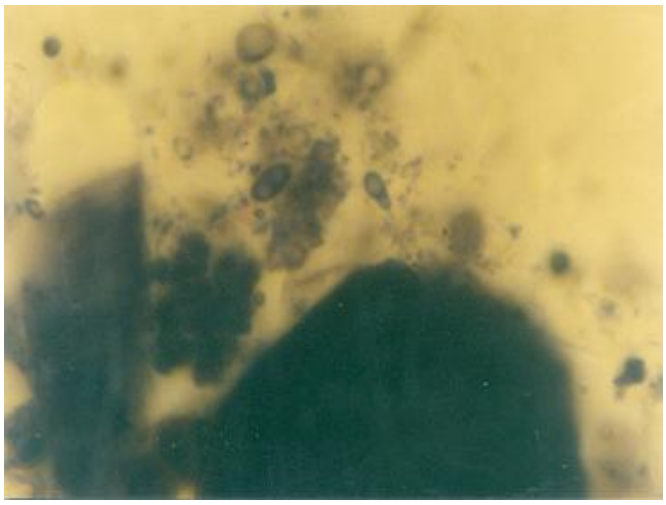

Figure 23. The bough of thallic growth of Figure 20, c, showing yeast cells in singles, doubles and clusters at 2000x magnification.

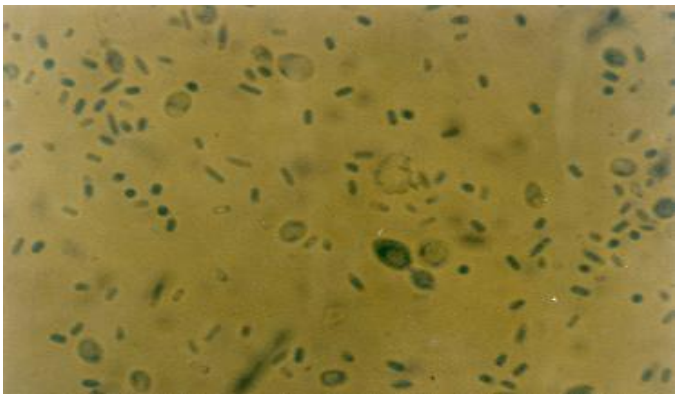

Figure 24. Terminal budding yeast cells of *M. circinelloides* Tieghem, which were predominant, induced in 1.0 g.l K^+- mediated and 0.10 g/l Na^+- modulated multiionic broth at 2000x magnification.

When modulation was with 0.15 g/l Na^+, the observed morphologies included septate hyphae with vesicular conidial head groups, holothallic conidia, holoblastic conidia, enterothallic conidia and growth spheres (Figure 25). Although terminal budding yeast cells were present, they were scanty (Figure 26). But numerous were enlarging protoplasts (out of focus in Figure 27), which could be in short rods (Figure 28) and bacteria- like. When growth spheres ruptured, protoplasts were released into the medium (Figure 29). They could also be extruded from hyphal cell after cell wall rupture. Figure 30 shows a filament with branch hyphae; as shown in Figure 31, one lateral branch has had its wall ruptured, and protoplasts released. This indicated that nucleates could convert to protoplasts even within the cytosol of an intact hyphal cell. Figure 32 shows thallic growth, conidium, dispersed nucleates/protoplasts in the same field range. After cell wall rupture, the hyphal fragments or conidia collapsed, becoming fragile (Figures 33 and 34).

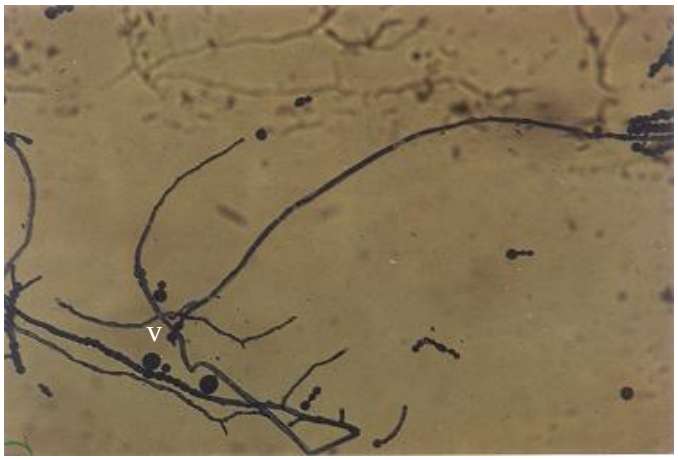

Figure 25. Holothallic-, holoblastic- and enterothallic- conidia of *M. circinelloides* Tieghem induced in 1.00 g/l K^+- mediated and 0.15 g/l Na^+- modulated multiionic broth at 200 x magnification.

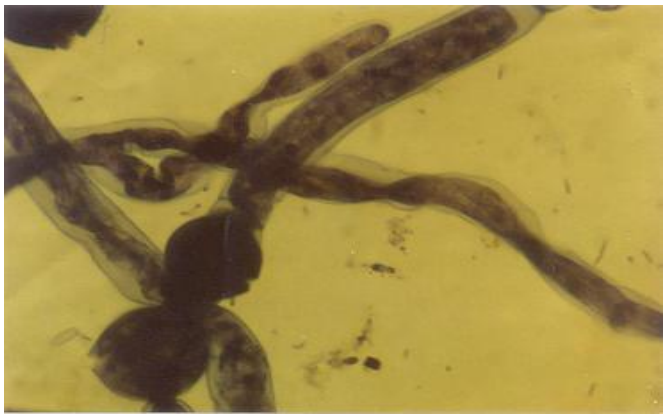

Figure 26. A section of Figure 25-marked 'v' showing ruptured growth sphere, septate hyphae and terminal budding yeast cells at 2000 x magnification.

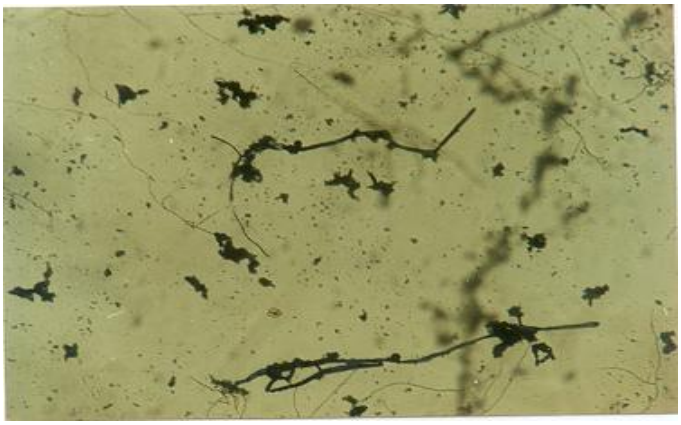

Figure 27. Thallic, holothallic growth and protoplasts (out of focus) of *M. circinelloides* Tieghem induced in 1.00 g/l K^+- mediated and 0.15 g/l Na^+- modulated multiionic broth at 200x magnification.

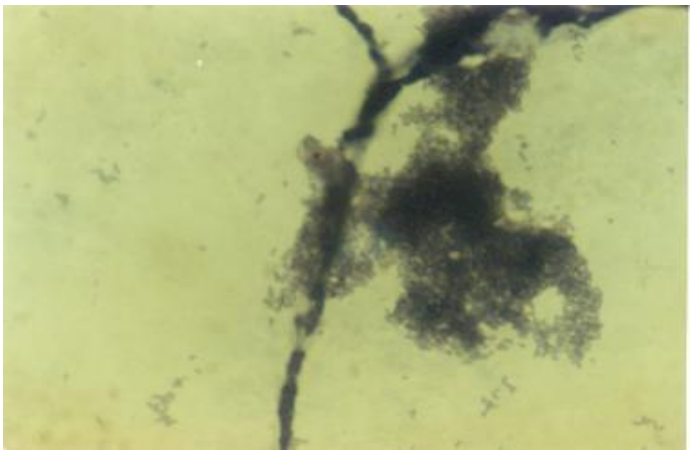

Figure 28. A view of Figure 27 showing fragile hyphae and protoplasts at 2000x magnification.

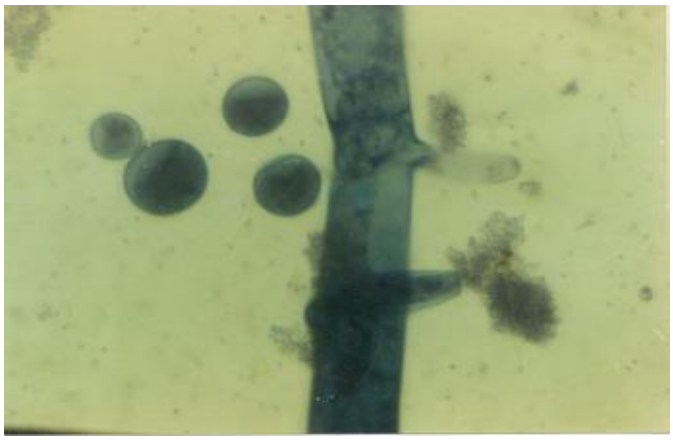

Figure 31. A section of Figure 30 marked 'w' showing growth spheres, blastic conidia, a segment of the thallic growth with tip-ruptured lateral branch and extruded protoplasts at 2000x magnification.

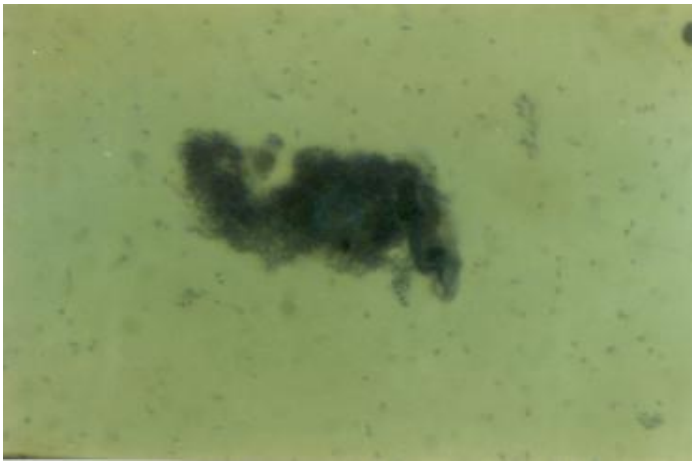

Figure 29. Granular units of *M. circinelloides* Tieghem released after growth sphere wall lyses in 1.00 g/l K^+- mediated and 0.15 g/l Na^+- modulated multiionic broth at 2000x magnification.

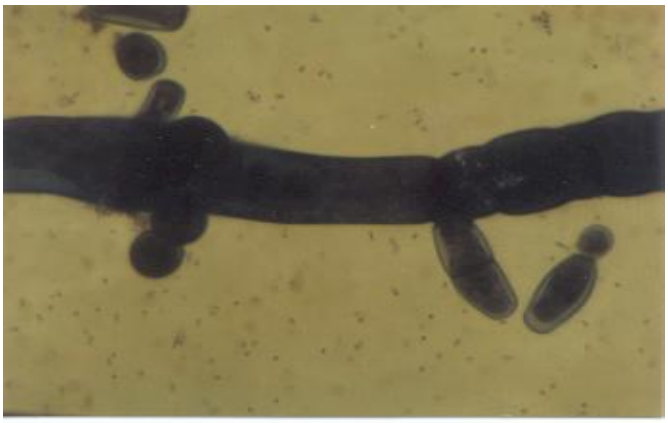

Figure 32. Segment of thallic growth which bud off enterothallic conidia after determinate growth; at the background are dispersed granular units of *M. circinelloides* Tieghem induced in 1.00 g/l K^+- mediated and 0.15 g/l Na^+- modulated multiionic broth at 2000x magnification.

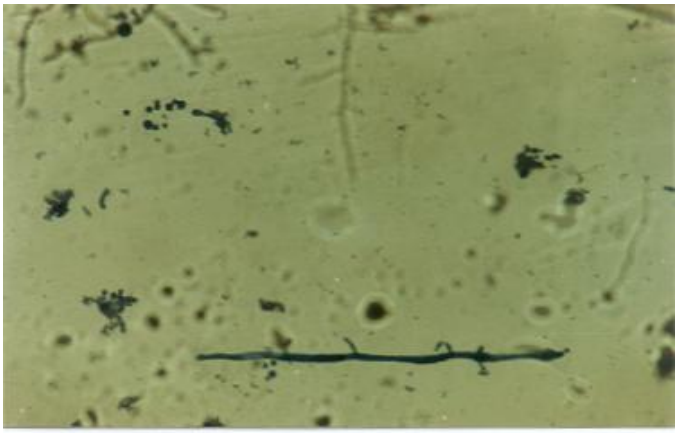

Figure 30. Clusters of growth spheres and short thallic growth of *M. circinelloides* Tieghem induced in 1.00 g/l K^+- mediated and 0.15 g/l Na^+- modulated multiionic broth at 200 x magnification.

At the 0.20 g/l Na^+ modulation, the septate hyphae, with vesicular conidial head groups were preponderant. So also were enterothallic conidia formed after determinate septate thallic growth. At low magnification (x200), they were very thin (Figure 35), in comparison with those at the lower Na^+ levels (Figures 25 to 27). Cytoplasm of thallic cells could become granular and might be released into the growth medium as granular units (Figure 36). Conidia from such thallic growth could be subglobose, oblong or rectangular (Figure 37). A meshwork of septate hyphae is shown in Figure 38.

Figure 39 is a section of Figure 38 showing hyphae with conspicuous septation. The same field range also showed growth spheres, holoblastic conidia and protoplasts. Figures 40 and 41 show the presence of enterothallic conidia of a different formation but surrounding the

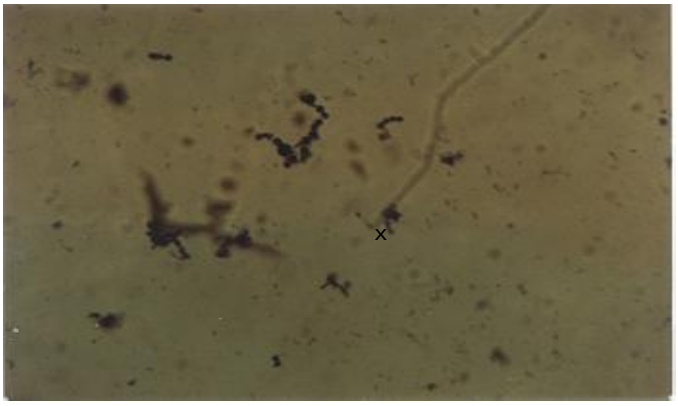

Figure 33. Clusters of holoblastic- and holothallic-conidia of *M. circinelloides* Tieghem induced in 1.00 g/l K^+- mediated and 0.15 g/l Na^+- modulated multiionic broth at 200x magnification.

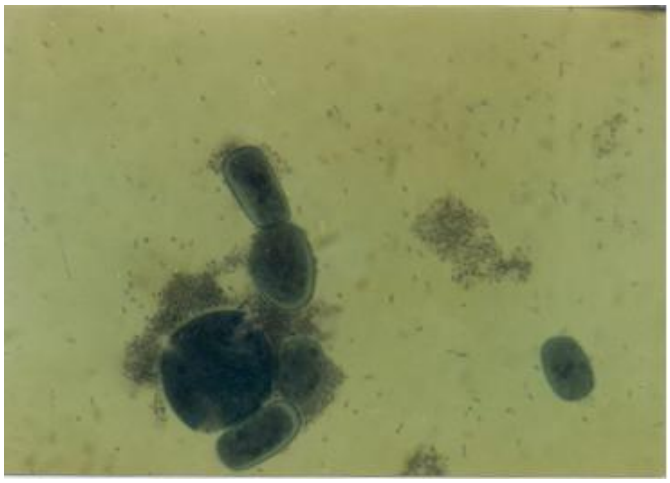

Figure 34. A section of Figure 33 marked 'x' showing the rupture of growth sphere which released its cytoplasmic contents as granular units and its subsequent collapse.

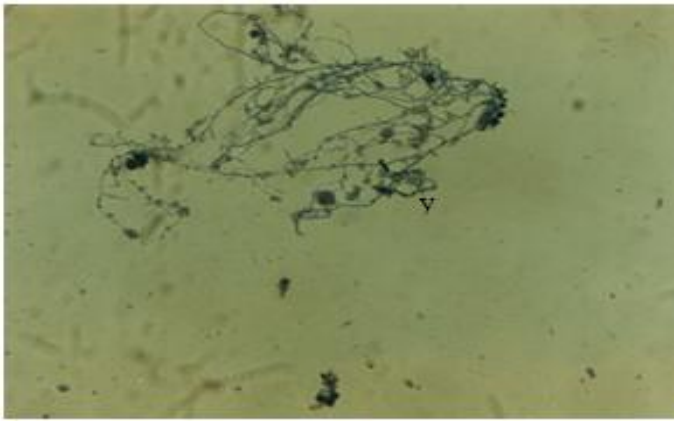

Figure 35. Thin fragile filaments of *M. circinelloides* Tieghem induced in 1.00 g/l K^+-mediated and 0.20 g/l Na^+- modulated multiionic broth at 200 x magnification.

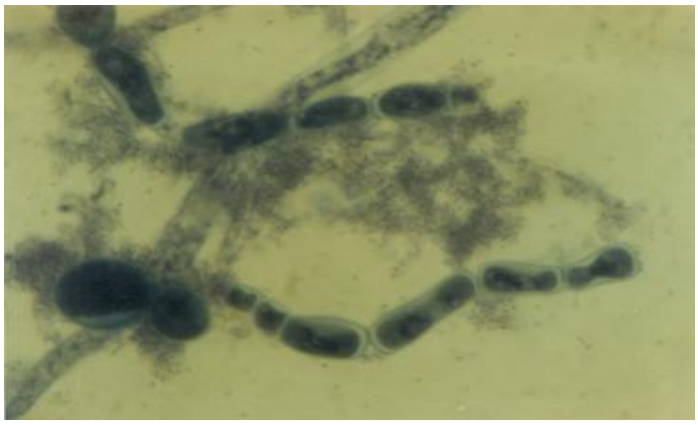

Figure 36. A section of Figure 35 marked 'y' showing enterothallic conidia, septate hyphal cell granular cytoplasm and 'released' granular particles of *M. circinelloides* Tieghem induced in 1.00 g/l K^+- mediated and 0.20 g/l Na^+- modulated multiionic broth at 2000x magnification.

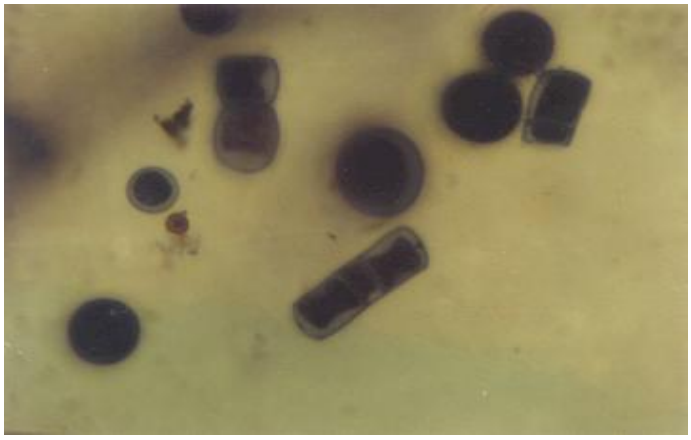

Figure 37. Globose, oblong and rectangular conidia of *M. circinelloides* Tieghem induced in 1.00 g/l K^+- mediated and 0.20 g/l Na^+- modulated multiionic broth at 2000x magnification.

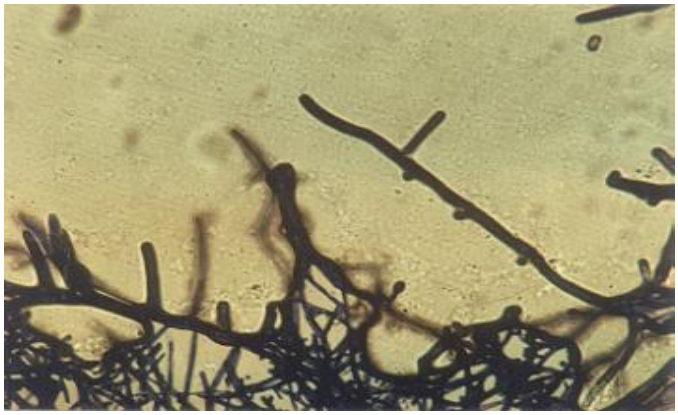

Figure 38. A meshwork of mycelia of *M. circinelloides* Tieghem induced in 1.00 g/l K^+- mediated and 0.20 g/l Na^+- modulated multiionic broth at 400 x magnification.

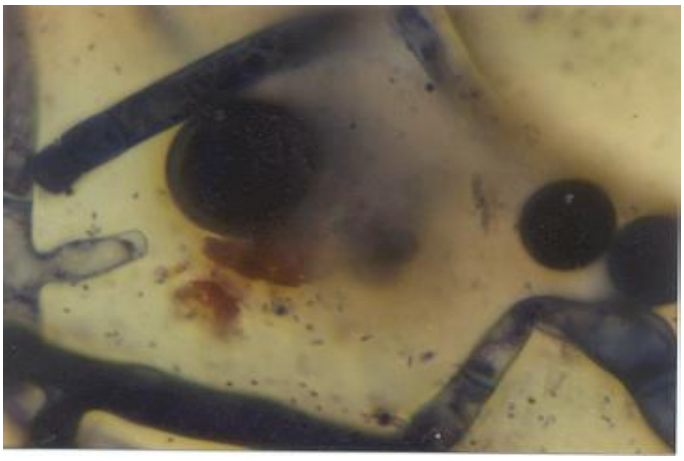

Figure 39. A section of Figure 38 showing septate hypha, holoblastic conidia and emergent yeast cells at 2000x magnification.

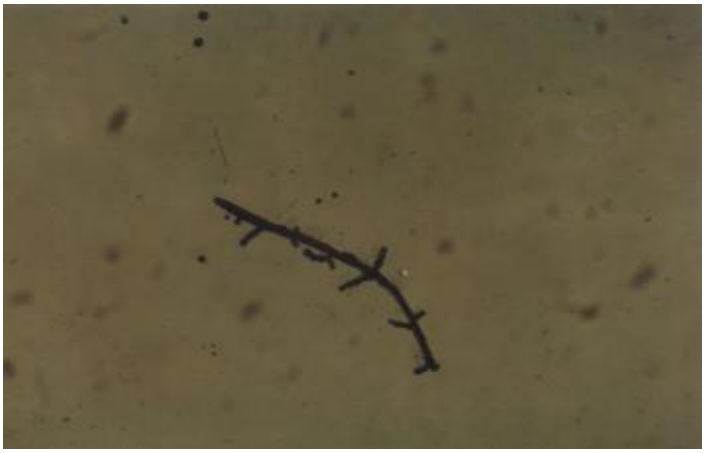

Figure 40. Determinate thallic growth of *M. circinelloides* Tieghem induced in 1.00 g/l K^+- mediated and 0.20 g/l Na^+- modulated multiionic broth at 200 x magnification.

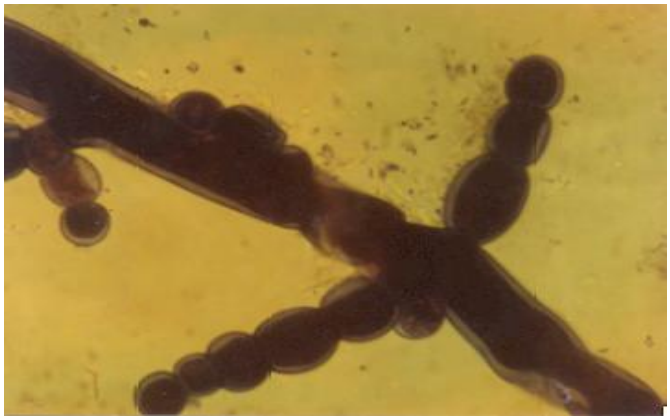

Figure 41. A segment of Figure 40 showing enterothallic conidia and emergent yeast cells at 2000x magnification.

Figure 42. Septate hyphae, growth spheres, holoblastic conidia and yeast cells (seen as granular units at the background) of *M. circinelloides* Tieghe induced in 1.00 g/l K^+- mediated and 0.20 g/l Na^+- modulated multiionic broth at 200x magnification.

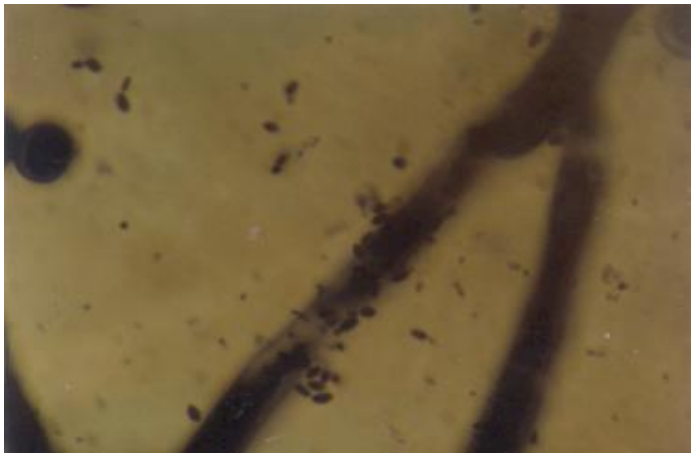

Figure 43. A segment of Figure 42 showing septate hyphae and yeast cells of *M. circinelloides* Tieghem induced in 1.00 g/l K^+- mediated and 0.20 g/l Na^+- modulated multiionic broth at 2000x magnification.

branches is emergent yeast cells. The protoplasts could also be globose or drum- like. Although, yeast cells occurred (Figures 42 and 43), they were not as numerous as the same morphology in the 0.10 g/l Na^+- modulated medium. Observation also showed that it was however more than the level that was induced in the 0.15 g/l Na^+- modulated broth.

The 1.10g/l K^+- mediated and 0.05 to 0.20g/l Na^+- modulated broths

The frequency of occurrence of the different types of morphology in the 1.10 g/l K^+- mediation diminished in comparison with the lower concentrations of K^+. When modulation was with 0.05 g/l Na^+, the thin walled septate hyphae with vesicular conidial head groups further

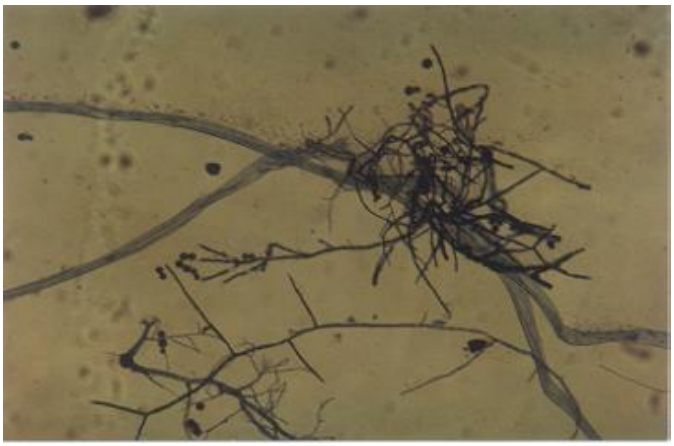

Figure 44. Growth spheres, holoblastic- holothallic- and enterothallic-conidia of *M. circinelloides* Tieghem induced in 1.10 g/l K^+- mediated and 0.05 g/l Na^+- modulated multiionic broth at 200 x magnification.

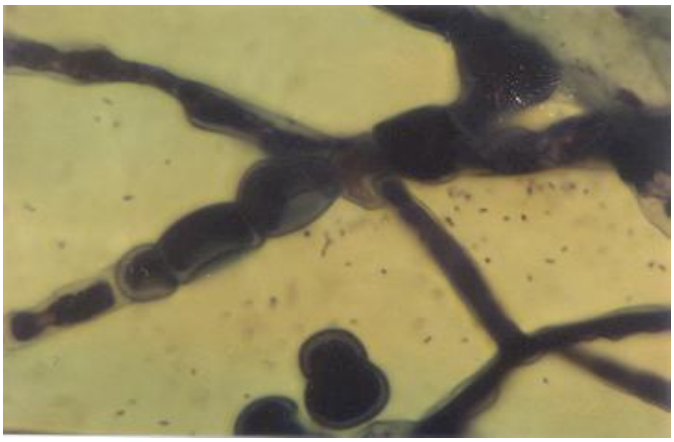

Figure 45. A segment of Figure 44 showing enterothallic conidia protoplasts and emergent yeast cells at 1000x magnification.

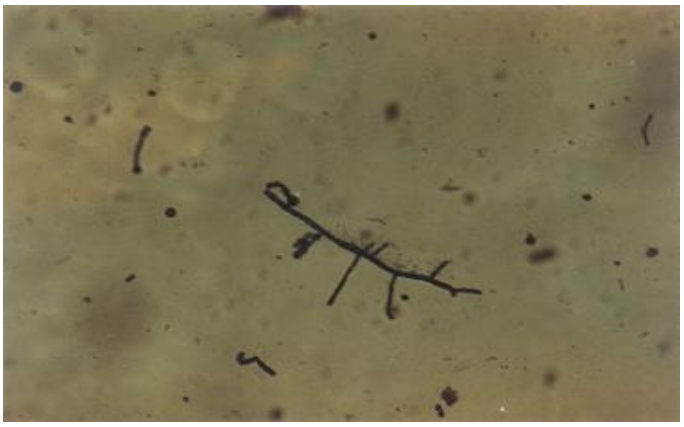

Figure 46. Isolated mycelia, growth spheres, holoblastic- and holothallic-conidia of *M. circinelloides* Tieghem induced in 1.10 g/l K^+- mediated and 0.10 g/l Na^+- modulated multiionic broth at 200x magnification.

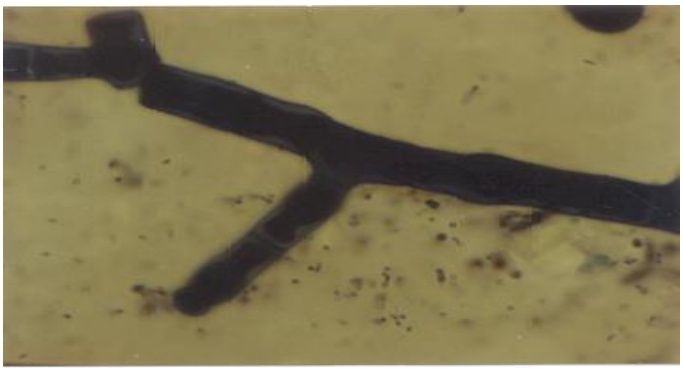

Figure 47. A segment of Figure 46 showing septate hyphae and protoplasts at 1000x magnification.

atrophied in contrast to the same morphology at the lower K^+ levels, and with that in control experiments as standard. Also co-induced were growth spheres, filaments, holoblastic-, and holothallic conidia (Figure 44). At higher magnification (x1000), the thallic growth was observed to develop into enterothallic conidia, but protoplasts, which were globose, subglobose, or rod- shaped (bacteria-like) were observed, although infrequently (Figures 44 and 45).

Germlines, as well as olive-green, thin-walled septate hyphae with vesicular conidial head groups and enterothallic conidia were co- induced with emergent yeast cells, when the medium was modulated with 0.10 g/l Na^+. Figure 46 shows isolated mycelia with the growth spore still attached (x200). At higher magnification, the septate hyphae were observed to break off at irregular intervals. Figure 47 shows these conidia (produced by fragmentation) in the midst of numerous protoplasts, which were globose, subglobose or rod- shaped. In this medium, the bacteria- like protoplasts were observed to assume greater sizes than those in the preceding Na^+ levels.

Septate hyphae with vesicular conidial head groups, holothallic-, holoblastic- and enterothallic conidia as well as scanty granular units were induced in the 0.15 g/l Na^+-modulated broths. Isolated hyphae with lateral branches were observed but these did not develop into mycelia (Figure 48). Higher magnification (x1000), showed conspicuous septation and the presence of granular units (Figure 49). At the highest level of Na^+-modulation (0.20 g/l), the morphologies were also scanty. Figure 50 shows the occurrence of enterothallic conidia (x200). Higher magnification (x1000) revealed the presence of yeast cells (Figure 51). The yeast cells were globose, subglobose and cylindrical, but also occurred as singles and doubles (Figure 52).

DISCUSSION

Chemical potential, Na^+ influx rate and cooperativity

An earlier study showed that the bulk medium pH level

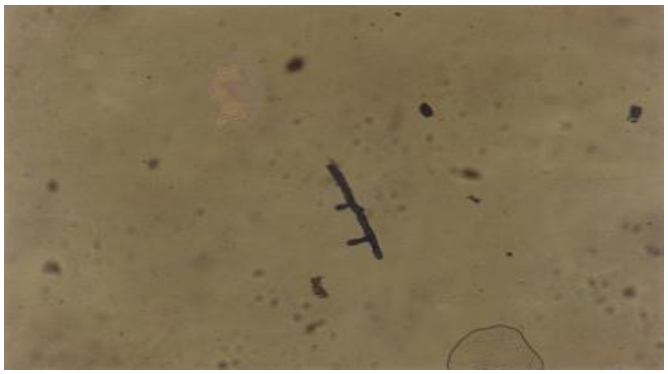

Figure 48. Isolated hypha of *M. circinelloides* Tieghem induced in 1.10 g/l K^+- mediated and 0.15 g/l Na^+- modulated multiionic broth at 200x magnification.

Figure 49. A section of Figure 48 showing septate hypha with lateral branches; observe the clusters of granular units (out of focus).

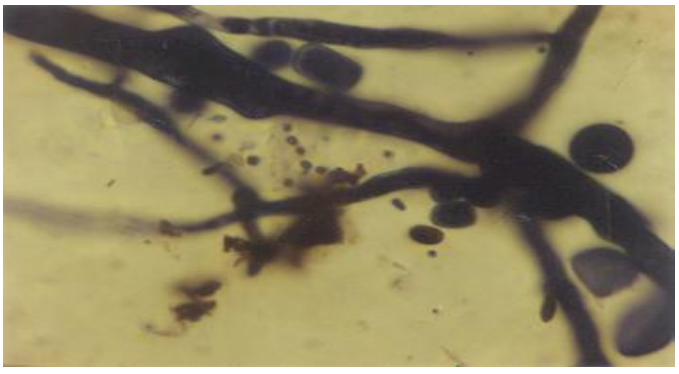

Figure 50. Septate hyphae and conidia of *M. circinelloides* Tieghem induced in 1.10 g/l K^+- mediated and 0.20 g/l Na^+- modulated multiionic broth at 200 x magnification.

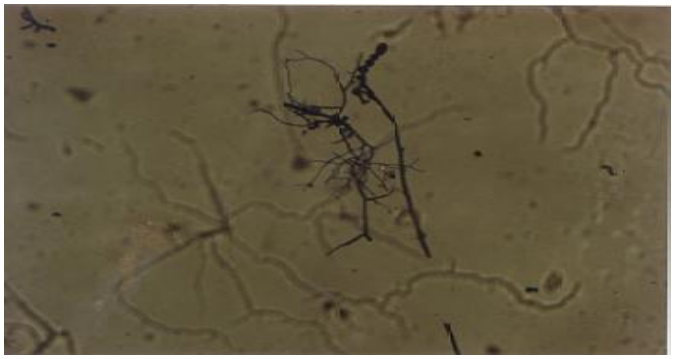

Figure 51. A segment of Figure 50 showing septate hyphae, conidia and emergent yeast cells 2000x magnification.

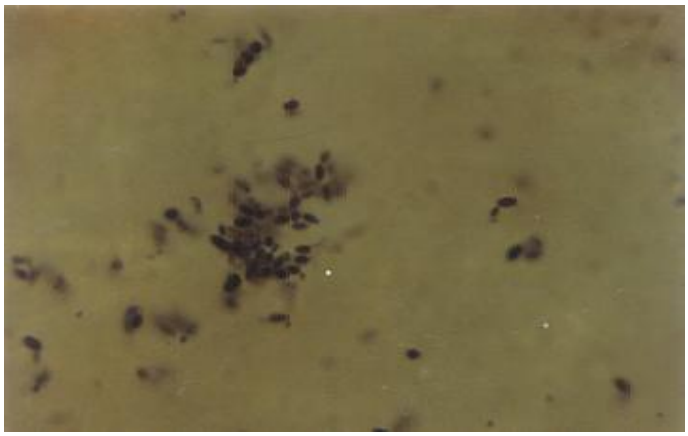

Figure 52. Terminal budding yeast cells of *M. circinelloides* Tieghem induced in 1.10 g/l K^+- mediated and 0.20 g/l Na^+- modulated multiionic broth at 2000x magnification.

was continuously changing as a result of flux of H^+ from the intracellular medium during the growth of the tentative form genus, *Dimorphomyces pleomorphis* (1996b). Similar observation has been made in the growth of *M. circinelloides* Tieghem (preceding report). The exchange of H^+ was attributed to the setting up of a transmembrane-pH-gradient, catalyzed by P- type H^+-ATPase (Albert et al., 1994; Tonomura, 1986). It was also shown that the intensity of H^+ release from the intracellular medium varied with the level of cations, specifically K^+, in the bulk medium and this affected the growth, on the one hand, and the ability to transform to terminal budding yeast cells, on the other, by sporangiospores of the filamentous microorganism, *M. circinelloides* Tieghem (preceding report). The present study confirmed these results. It also showed that Na^+ simultaneously incorporated with K^+ into the bulk medium affected the growth and transformability of sporangiospores to terminal budding yeast cells.

The establishment of transmembrane-pH-gradient (Omoifo, 1996b, 2003), as seen in the mitochondria, created anisotropic growth environment whereby ionic movement through the membrane was unidirectional (Abeles et al., 1972; Albert et al., 1994; Mitchel, 1967; West and Mitchel, 1972; Lehninger, 1975; Slayman and Slayman, 1974; Voet and Voet, 1995). This was amply demonstrated in this study. Ionic gradients therefore, also existed for Na^+ and K^+, thus providing the driving force for

such transductive energy movements. Hence Na^+ and K^+ were continuously being exchanged between the organism's intracellular medium and the bulk medium. Since the magnitude of the driving force would depend on the ionic charge and, hence vary from one level to the other, it was possible to obtain, from the rate of efflux of Na^+ at a given ionic charge, the chemical potential, *m,* of such ion. The momentum could be determined by the level of fall of internal concentration of an ion through a linear distance and in this case, represented by the time differential for such a fall. Figure 53a to c illustrated this for Na^+.

Observation showed that the period under consideration embraced several structural activities including the conversion of sporangiospores to growth spheres, conversion of inherent cytoplasm to granular particles/ cytosolic nucleates, lyses of the cellular envelop and subsequent conversion of nucleates to protoplasts. The value determined at each set of bulk medium K^+- Na^+ treatment could represent the magnitude of force required for isotropic growth and hence osmotic work, lytic activity on cell wall and plasma membrane destruction, and cytosolic nucleation (this represents the formation of cytoplasmic granular units). The interesting results obtained from such analysis showed that this was generally higher at the 0.9 g/l K^+- and lower at 1.0 g/l K^+ mediation. But the lowest value of *m* was obtained at 1.10 g/l K^+-0.20 g/l Na^+ ionic charge.

The continuous flux of K^+ and Na^+ through the membrane was oppositely unidirectional. When plots of Na^+ versus Na^+/K^+ were obtained at each bulk medium ionic charge, the plots were highly associative (Figure 54a to i), except for the higher levels of bulk medium ionic charge (Figure 54j to l). Such relationship was most cryptic with K^+ versus Na^+/K^+ plots (Figure 55a to l). These graphs demonstrated that the active flux of these ions were interdependent. This possibly meant that a shift in the level of K^+ entering the intracellular medium also affected the level of Na^+ extruded. Thus, it appeared that the permeability of these electrolytes through the membrane was intimately linked, but was also controlled by the same electrostatic forces (Diamond, 1975), which in turn, varied from one bulk medium Na^+ versus K^+ ionic charge to the other. Perhaps, this was attributable to the differences in ionic strength at the different levels of bulk medium ionic charge.

The membrane-bound transport protein, Na^+ and K^+-ATPase, which has antiport function for Na^+ and K^+, mediates such linked transport mechanisms, in eukaryotes, with multidimensional effect on proliferation, differentiation and transformation (Abeles et al., 1992; Albert et al., 1994; Tonomura, 1986; Spark et al., 1982; Wright and Diamond, 1968). This antiport transport effect was demonstrated in this study. Thus, apart from the assumed P-type, H^+- ATPase, which is responsible for the H^+-pump mechanism, it was also strongly suggested that the Na^+, K^+-ATPase was functional during the transformative activities leading to yeast formation from sporangiospores of *M. circinelloides* Tieghem, in this study. Experiments in our laboratory have shown that the highest growth magnitude of *M. circinelloides* Tieghem occurred at 1.0 g/l K^+ incorporation of the growth medium. This coincided with the 1.0 g/l K^+ mediation and 0.10 g/l Na^+ modulation in the present study. When the electrolytic fluxes were juxtaposed with the growth pattern at each ionic charge (Figure 56a to l), the trend lines for Na^+ flux within the first 72 h of growth depicted periodic efflux followed by influx, except for Figure 56d and k, respectively efflux at 48 to 72 h and influx at 24 to 48 h-growth periods. Since Figure 56f exhibited valid sigmoid growth curve, which had a Na^+ extrusion phase that coincided with the lag phase, and the Na^+ accumulation phase that coincided with the exponential phase, it meant that a specific parameter, *x*, for Na^+ influx rate, could be derived for comparative purposes. This is shown in Figure 57.

The chemical potential (*m*) of 1.05625 with a correspondingly high Na^+ influx rate, (*x*, 1.327083), did not induce yeast cell. Yeast cells were induced at half of the value of *m* (0.5625) with similarly low *x* (0.064583), and copiously too. An increased magnitude of *m* (1.354166667) but with a low *x* (0.072917) induced yeast cells although scantily, and they were only just emergent yeast cells, which never developed to the matured yeast state of the preceding configuration. When *m* was about half the magnitude obtained at 0.10 g/l Na^+ concentration, but at the lowest influx rate (*x,* -0.21458), the emergent yeast cells were hardly visible.

It was inferred from this analysis that a measure of sequential cooperativity existed between the chemical momentum or ionic potential and the influx rate of Na^+ that led to the induction of the yeast morphology. Intrinsic structural changes that occurred in this transformative process included cytosolic nucleation and protoplast formation. Since protoplasts were not formed in K^+-deficient medium (preceding reports), it was thought that K^+ was absolutely necessary in the biochemical reactions leading to cytosolic nucleation with subsequent protoplast formation. The yeast induction varied in emergence, preponderance, maturity, size and shape from one ionic charge to the other suggesting that Na^+ and K^+ sought balance that promoted the greater probability for the transformative process. When the concentrations reached the right value, yeast cells emerged. This could be referred to as the critical concentration, *cc*. The *cc,* which had the strongest degree of cooperativity, would therefore give the most appropriate configurations of *m* and *x*, which promoted the transformation to the yeast morphology.

Since the highest conformational transformation occurred at 1.0 g/l K^+ and 0.10 g/l Na^+ ionic charge, this constituted the perfect *cc,* with the *m* and *x* configuration being 0.104166667 and 0.472917, respectively. These cooperative interactions amplified transformative activities

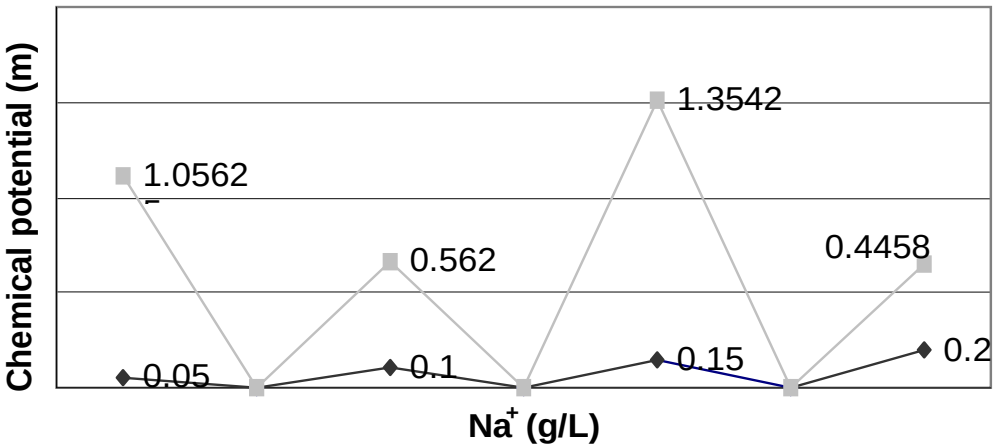

Figure 53a. Effect of Na^+ incorporation on momentum (m) of ionic movement in an anisotropic environment mediated by 0.9 g/l K^+ during the cultivation of *M. circinelloides* Tieghem in glucose-substrate multiionic broth.

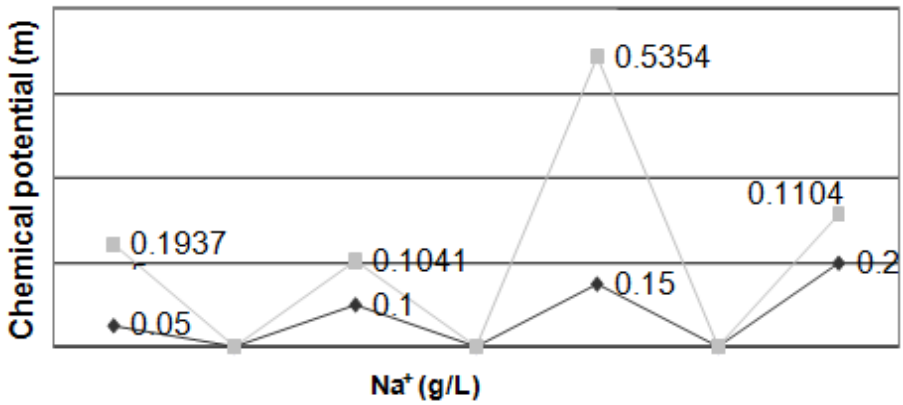

Figure 53b. Effect of Na^+ incorporation on momentum (m) of ionic movement in an anisotropic environment mediated by 1.0 g/l K^+ during the cultivation of *M. circinelloides* Tieghem in glucose-substrate multiionic broth.

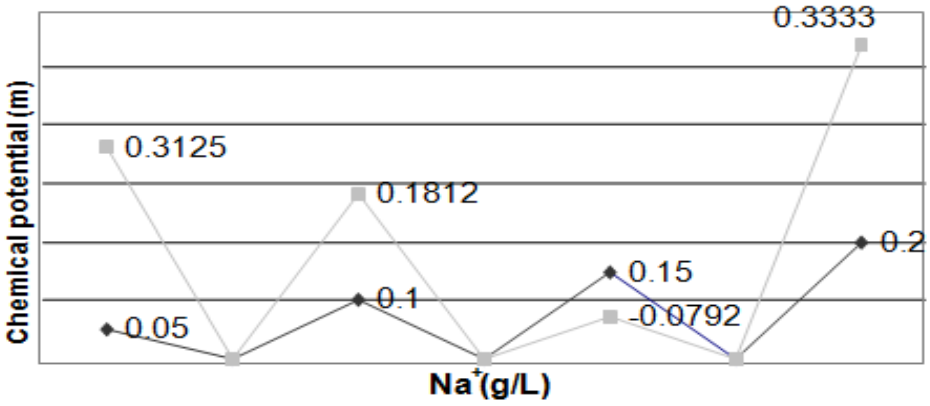

Figure 53c. Effect of Na^+ incorporation on momentum (m) of ionic movement in an anisotropic environment mediated by 1.10 g/l K^+ during the cultivation of *M. circinelloides* Tieghem in glucose-substrate multiionic broth.

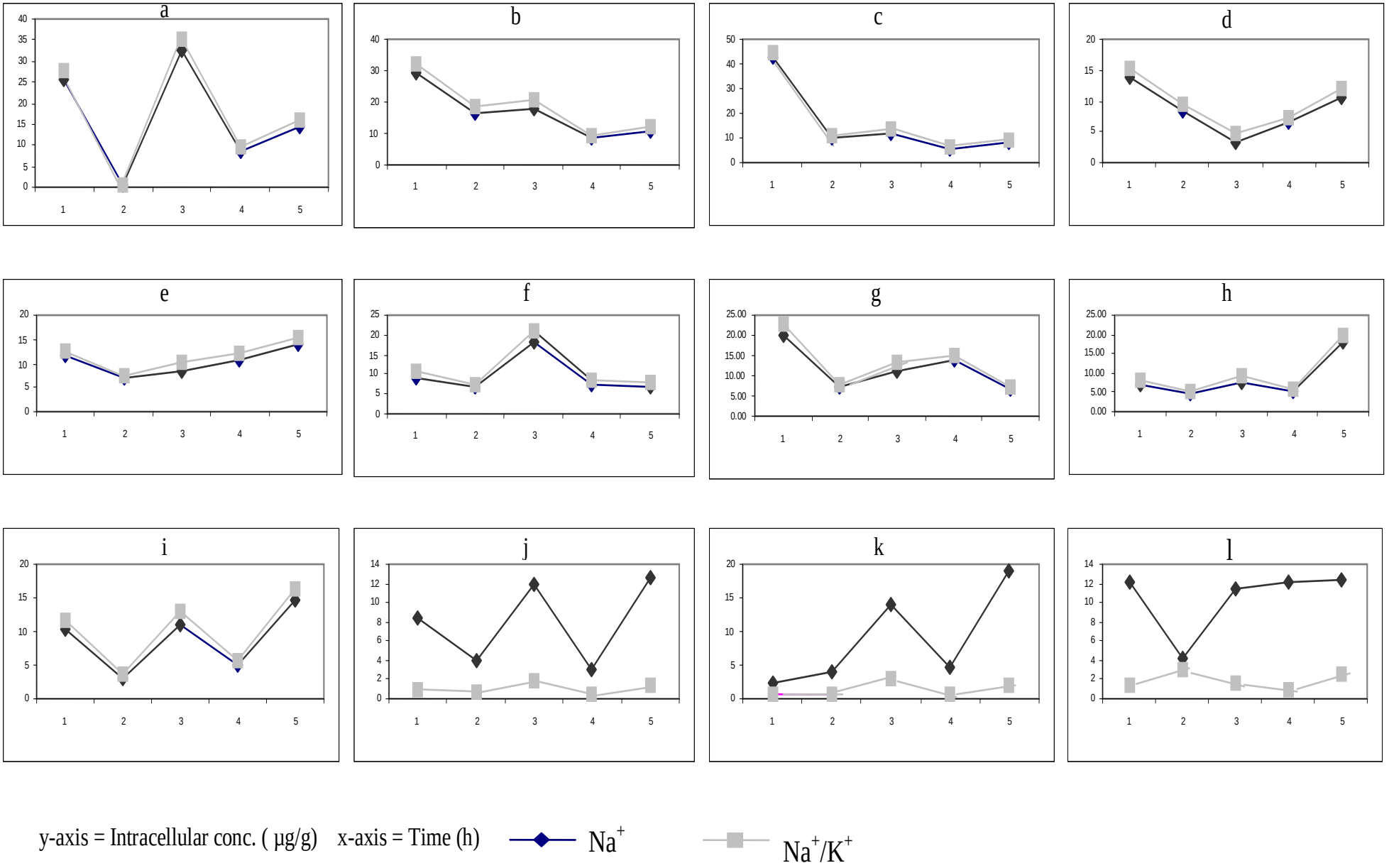

Figure 54. a to l: Charts showing flux of Na^+ versus Na^+/K^+ in the intracellular medium during sporangiospore-yeast transformation of *M. circinelloides* Tieghem cultivated in buffered glucose-substrate multiionic broth incubated for 120 h at pH 4.5, temperature of 20°C (ambient).

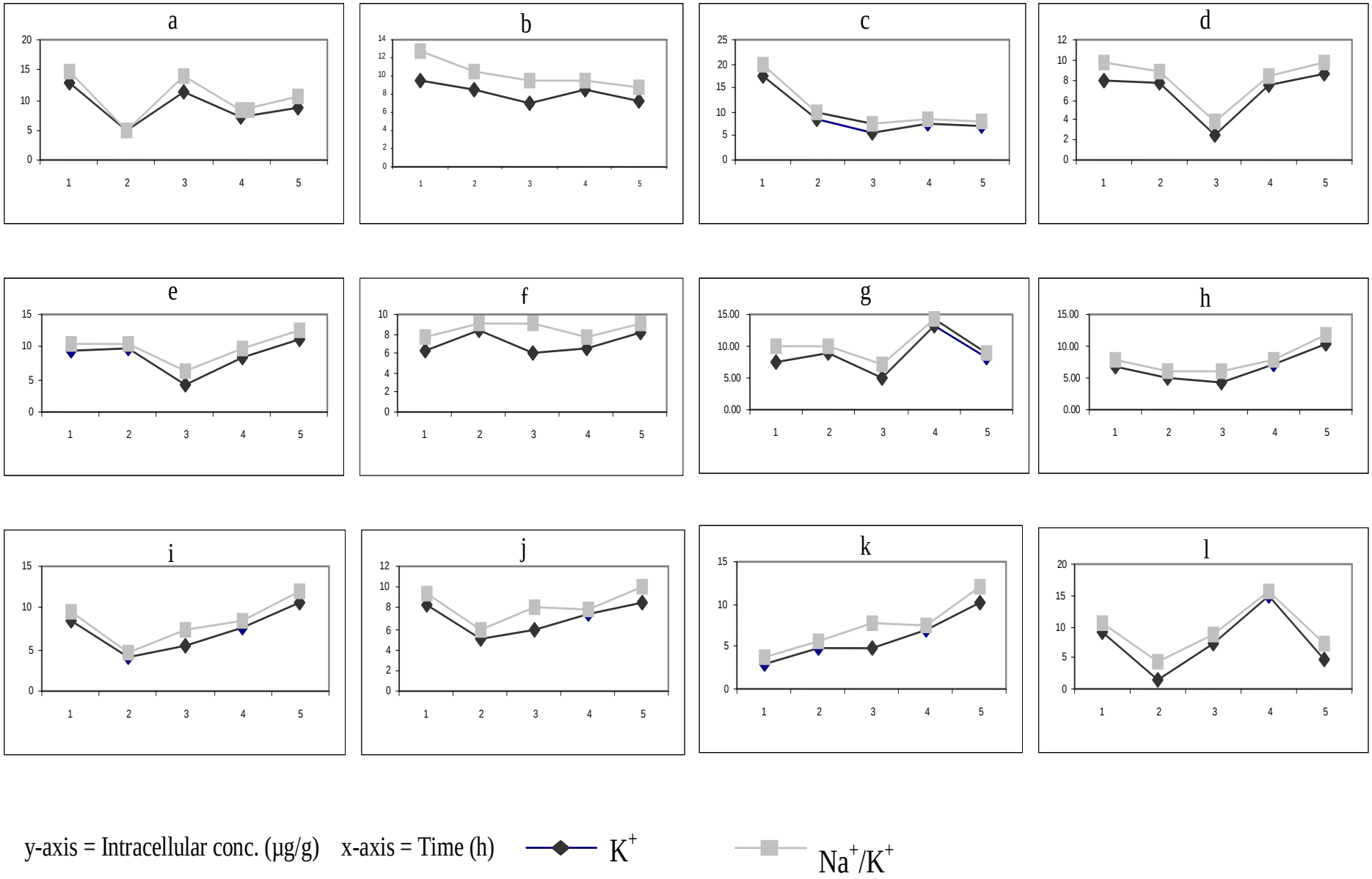

Figure 55. a to l: Charts showing flux of K^+ versus Na^+/K^+ in the intracellular medium during sporangiospore-yeast transformation of *M. circinelloides* Tieghem cultivated in buffered glucose-substrate multiionic broth incubated for 120 h at pH 4.5, temperature 20°C (ambient).

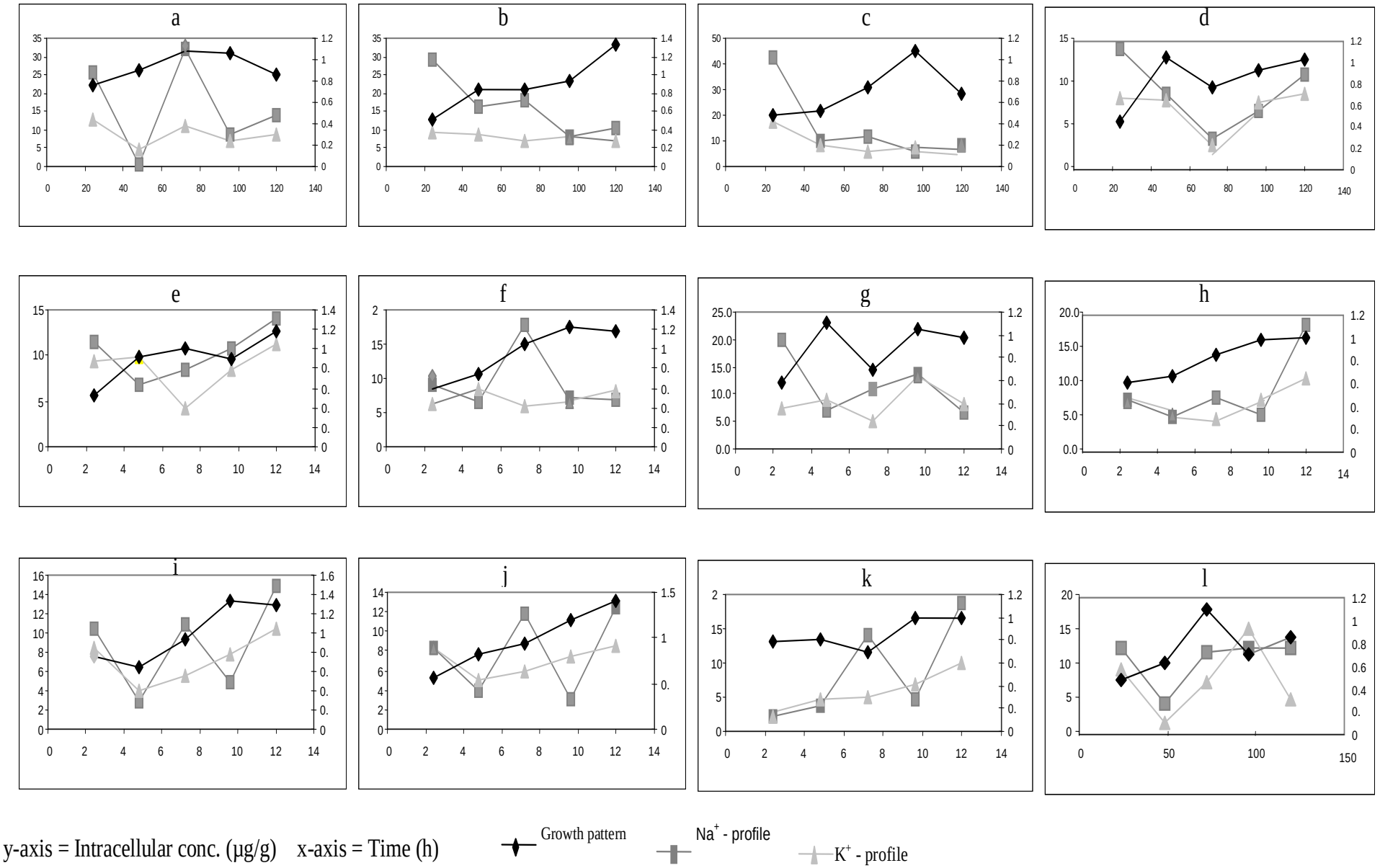

Figure 56. a to l: Growth patterns and intracellular Na^+ and K^+ variation during the growth of *M. circinelloides* Tieghem cultivated in buffered glucose-substrate multiionic broth for 120 h at pH 4.5, temperature 20°C (ambient).

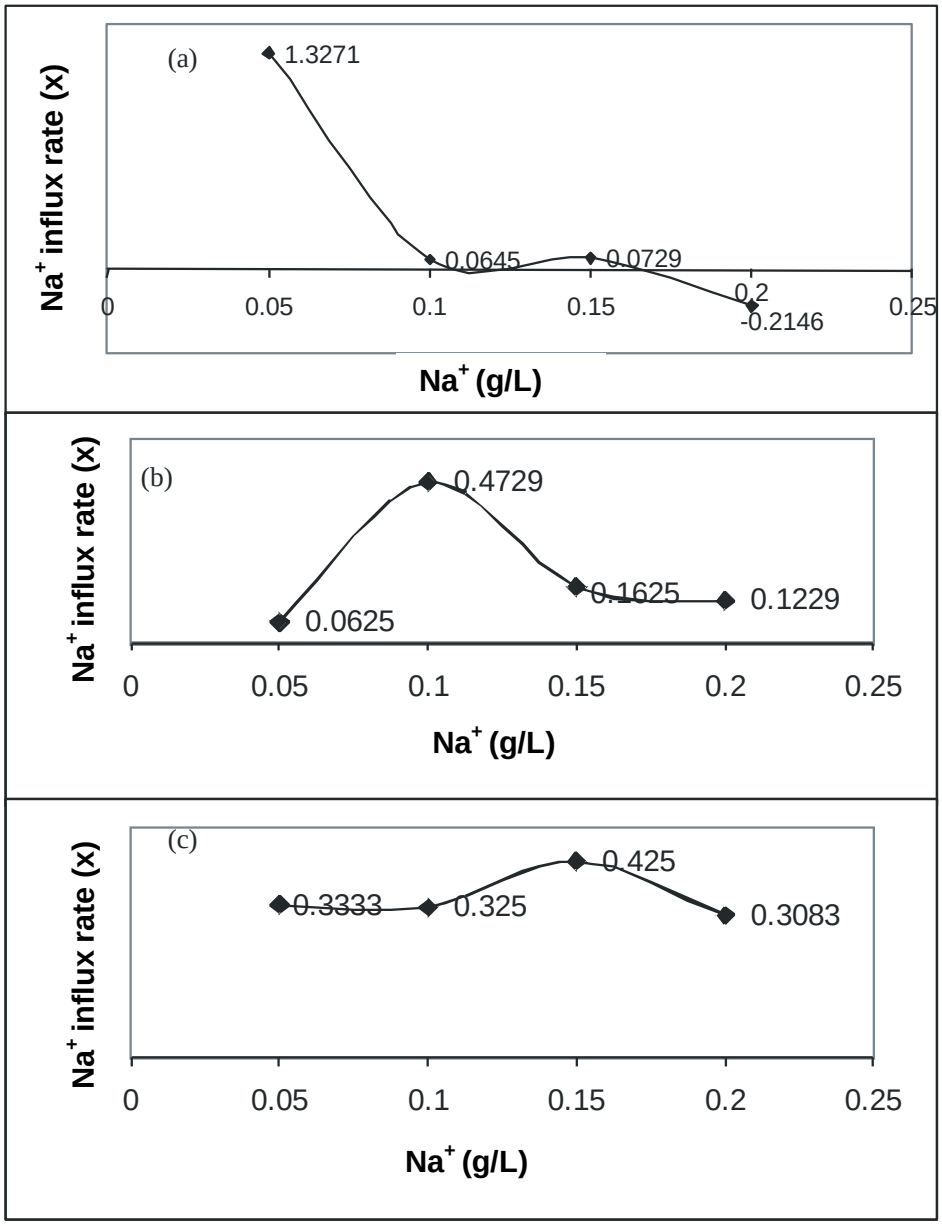

Figure 57. Specific influx rate of Na^+ into intracellular medium at exponential growth phase of *M. circinelloides* Tieghem when cultivated in glucose-substrate multiionic broth mediated with (a), 0.90 (b), 1.00 and (c), 1.10 g/l K^+.

that permitted perfect sigmoid curve. A functional linkage was therefore implicated through these significant parameters, *cc*, *m* and *x,* limiting the particularizing of the intrinsic structural changes leading to the yeast form.

The significance of the cc of ions, *m* and the *x* in the transformation to yeast cells could be further appreciated when Na^+ concentration was varied within the same level of K^+ charge. At the 1.0 g/l K^+-mediated broth, while sigmoid curves were obtained with 0.10 and 0.20 g/l Na^+ charge, the former permitted a higher level of growth (*sp.g*, 0.012604) than the latter (*sp.g*, 0.008125). This meant that there was faster rate of yeast formation in the former. This was in spite of the fact that *m* for the latter was higher by 0.0006249987 units. Such higher momentum permitted a lower level of *x* (0.122917) than the former (*x*, 0.472917). It therefore meant that there was greater cooperativity with the configuration at 1.0 g/l K^+-0.10 g/l Na^+ than the 1.0 g/l K^+-0.20 g/l Na^+ bulk medium.

The major observable events in the growth sphere included cytosolic nucleation and cell envelope destruction. It appeared that the less cooperative a system was, the greater the chemical potential required for accomplishing these tasks. This was illustrated by the high *m*-value at the different K^+- charges. At 0.9 g/l K^+-0.05 g/l

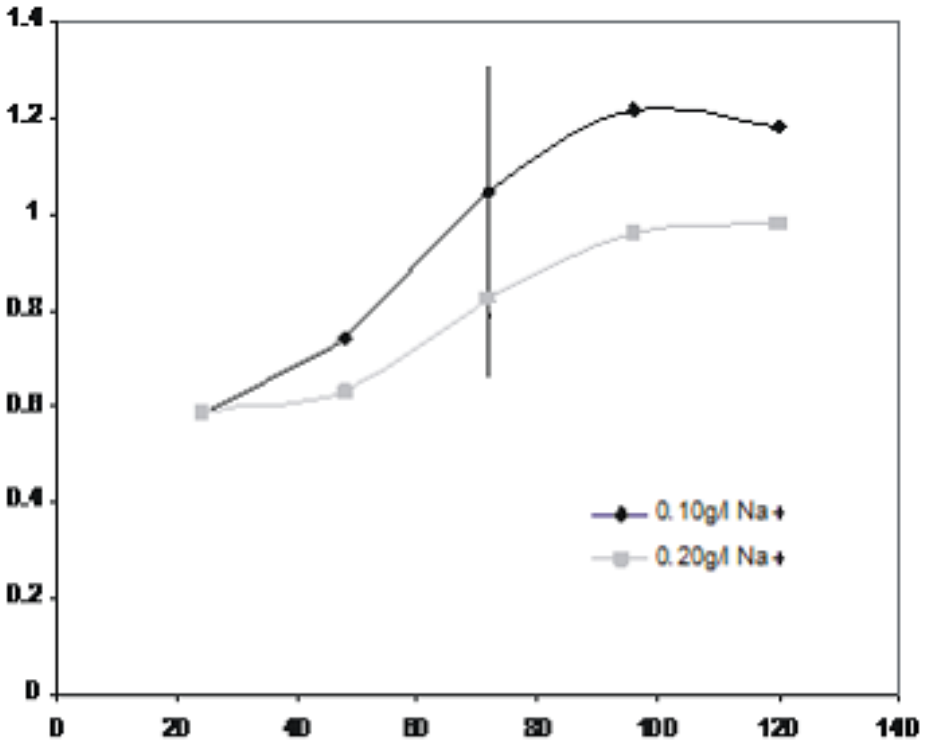

Figure 58. Sigmoid curve and Na^+ influx rate at exponential growth phase of induced yeast cells of *M. circinelloides* Tieghem cultivated in broth charged with 1.0g/l K^+. X=Na^+ influx rate.

Na^+, high *m* (1.05625) was required to transit some growth spheres to cytosolic nucleates, and protoplasts, while tubular growth effused from the others. But yeast cells were not induced. At 1.0 g/l K^+-0.05 g/l Na^+, a lower *m* (0.19375) promoted nucleation, protoplast formation and the transformation to yeast cells. This showed some degree of cooperativity. However it was not very high as manifested by the lack of sigmoid growth pattern; furthermore, thallic growth and conidial expression dominated the medium. When the Na^+ charge was increased to 0.10 g/l at the same K^+ concentration, a still lower *m* (0.104166667) was derived and this endorsed sigmoid growth pattern, a high cooperativity system manifesting principally the yeast morphology. But at 1.10 g/l K^+-0.05 g/l Na^+, no yeast cell was formed, in spite of the moderate chemical potential, (*m, 0.3125*). That the modest chemical potential coupled with an *x* of 0.3333 was not enough to induce yeast cells showed that the system was non-cooperative for yeast formation. Figure 58 showed that the Na^+ influx rate was high for the 0.90 g/l K^+-0.05 g/l Na^+ (*x*, 1.327083), comparatively low for the 1.10 g/l K^+-0.05 g/l Na^+ (*x,* 0.33333) and much lower for the 1.0 g/l K^+-0.05 g/l Na^+ charge (*x*, 0.0625), which was one of the systems that induced yeast cells, although it did not permit sigmoid growth curve, and hence, less cooperative in contrast to the 1.0 g/l K^+-0.10 g/l Na^+ charge, the valid system that induced principally yeast cells and exhibited sigmoid growth pattern. Therefore, at constant Na^+ charge, the K^+ concentration that will balance up such charge so as to trigger an appropriate Na^+ influx rate for yeast morphology induction, need to be carefully determined.

That nucleates and protoplasts occurred at 0.90 g/l K^+-0.05 g/l Na^+ charge but less frequently at 1.10 g/l K^+-0.05 g/l Na^+ charge, point out that excess K^+ could not be very advantageous to the biochemical activities leading to cytosolic nucleation or protoplast formation. When the Na^+ influx rate was too low, cooperative transition from protoplasts to yeast cells was near nil. In contrast to the preponderance of yeast cells in the 0.9 g/l K^+-0.10 g/l Na^+ charge (*x*, 0.064583) with the absence of yeast cells at the 0.90 g/l K^+-0.20 g/l Na^+ charge (*x*, -0.21458), protoplasts were induced scantily. Since the degree of Na^+ accumulation played vital role in the conversion of protoplasts to yeast cells, it would affect the steepness of the sigmoid curve. This is seen in the 1.0 g/l K^+ charge where modulation with 0.10 g/l Na^+ (*x*, 0.472917) had near perfect sigmoid description than that with 0.20 g/l Na^+ (*x*, 0.122917) (Figure 58).

Ionic circulation and morphogenesis

This study confirms the cryptic observations that led to the formulation of the sequential sporangiospore-yeast transformation (SSYT) hypothesis and further supports the implied role of ionic gradients through semi perme-

able membrane of growth sphere or protoplast proposed by Omoifo (2003). The congruence of observations of the present study with earlier studies therefore gives credence to the lateral morphogenetic transformability that has been identified with *Dimorphomyces diastaticus* strain C12, IMI W5132A (Omoifo, 2003, 2005) and *D. pleomorphis* strain C13, IMI W5132B (Omoifo, 1996b) and place the yeast cells derived thereof in the same inductive pathway. This means that sporangiospores of *M. circinelloides* Tieghem, *D. diastaticus* strain C12, IMI W5123A and *D. pleomorphis* strain C13, IMI W5123B were subjected to similar biophysical principles as well as undergoing the same structural modifications in order to convert to terminal budding yeast cells. However, obvious differences occurred in size and shape of this expressive morphology of one microorganism to the other.

Two effects resulted from buffering the bulk medium at acidic pH level. It afforded intrinsic direction in space for inter- and intracellular communication and imposed an imbalance of electrochemical potential of H^+ across the membrane. These made the cytoplasm more alkaline or electrically negative, and hence drive the circulation of H^+. In the cooperative system, it was thought that membrane-integrated primary transport systems, H^+-ATPase and Na^+ -ATPase, mediated inter-conversions between the free energy of chemical reactions and that of electrical potential or concentration gradients. Thus, the controlled utilization of ionic currents provided transductive energy, which performed physiological roles (Saprks et al., 1982; Skou, 1957), thus, causing the observed structural changes. Based on the observed ionic fluxes, the possible mode of behavior of H^+- and Na^+-pumps in the growth phases during the cooperative transformation of sporangiospores to yeast cells is shown in Figure 59.

Table 1 shows the ratio of intracellular Na^+ to K^+ concentration during the growth phases. During the lag phase in the functional cooperative system, 1.0 g/l K^+-0.10 g/l Na^+, efflux of 3.03 units of the intracellular Na^+ was concomitantly followed by influx of 2.2 units of K^+. Under normal physiological conditions, this would be coupled to the hydrolysis of 1 unit of ATP, in a system known to be electrogenic (Dixon and Hopkin, 1980). Observation showed that this was the period of isotropic growth; cytosolic nucleation and the accompanying cell envelop destruction. The possible mechanisms for these biochemical activities have been explained (Omoifo, 2003). It could therefore be stated that at critical internal concentrations of K^+ and Na^+, synchronous DNA synthesis occurred, utilizing the electrogenic energy generated, which subsequently led to cytosolic nucleation (granular particles in Omoifo, 1996b, 1997). When lyses of cell wall and disruption of the lipid membrane of the growth sphere occurred (Omoifo, 1996b, 1997, 2003; the present study), nucleates were released into the bulk medium. It was perceived that in these nucleates, the ratio of ADP + Pi to ATP increased as the ATP pool size ran down (Berns, 1977; Chin and Berstein, 1968), the aforementioned activities being energy demanding (Tonomura, 1986) and the concentration gradients of Na^+ and K^+ across the membrane is far steeper than the previous levels. Indeed Table 1 shows a 2.1:2.8 Na^+ to K^+ ratio in the intracellular medium at 48 h of growth. The reversal of chemical ratio was therefore considered a reflection of energy imbalance. This thus created an off state for the progressive replication of DNA and, hence nucleation. Furthermore, a reversal of direction in space of transport mechanism occurred, the electrochemical gradient of H^+ being used to import materials, specifically Na^+, is thought to regulate the activity of biosynthetic enzymes into the perhaps, non-protoplasmic nucleates. The continued operation of the Na^+-K^+ antiport system, which was also coupled to ATP synthesis, would perhaps guide biosynthetic enzymes along specific paths and could induce early genes and enzymes thereof that transited the primordial units to protoplasts. The pH gradient also drives the import of glucose (Alderman and Hofer, 1981; Reber et al., 1977). The H^+-substrate symporter system could ensure the availability of carbon substrate in the cytosol. Consequently, cellular metabolism could be driven in the direction of substrate-level phosphorylation. A study in our laboratory, using *Rhizopus stolonifer*, has led to similar interpretation. These transport events in a cooperative system, would enhance the probability of others. It was proposed that with the availability of ionic species and substrate in the cytosol, inherent conditions permitted second messenger generation, and thus triggered signal transduction leading to mitogenesis; this therefore would be occasion for physiological activities that give rise to yeast induction (Omoifo, 2003). Perhaps, this process occurred in the present study.

In this study, subglobose and obpyriform yeast cells which were terminal budding, developed. This was in a similar manner with *D. pleomorphis* strain C13, IMI W5132B, although globose and ellipsoidal yeast cells were induced (Omoifo, 1996b). Occurrence of branched filaments, albeit scanty and did not develop into meshwork, was predicated on the occurrence of spontaneous dismutation, thereby releasing superoxide, which on disproportionate by superoxide dismutase, yielded oxygen for intrinsic respiration, as was thought to be the case with *D. diastaticus* strain C12, IMI W5132A (Omoifo, 2003).

The biological clock of S*accharomyces cerevisiae* runs through four phases, viz: lag phase, when the organism adjusts to its food source; log phase, when there is rapid cell division and the organism increases at exponential rate; stationary phase, when the death rate is equivalent to the rate of formation of new cells; and the death phase, when the rate of programmed cell loss or apoptosis increases. During the latter phase, the nucleus condenses and cell shrinks. Sporangiospores transformed in our high cooperative system, nicely approximated this clock with a final morphological expression as yeasts.

Reference has been made to Na^+/K^+ ratio of 3.03:2.20

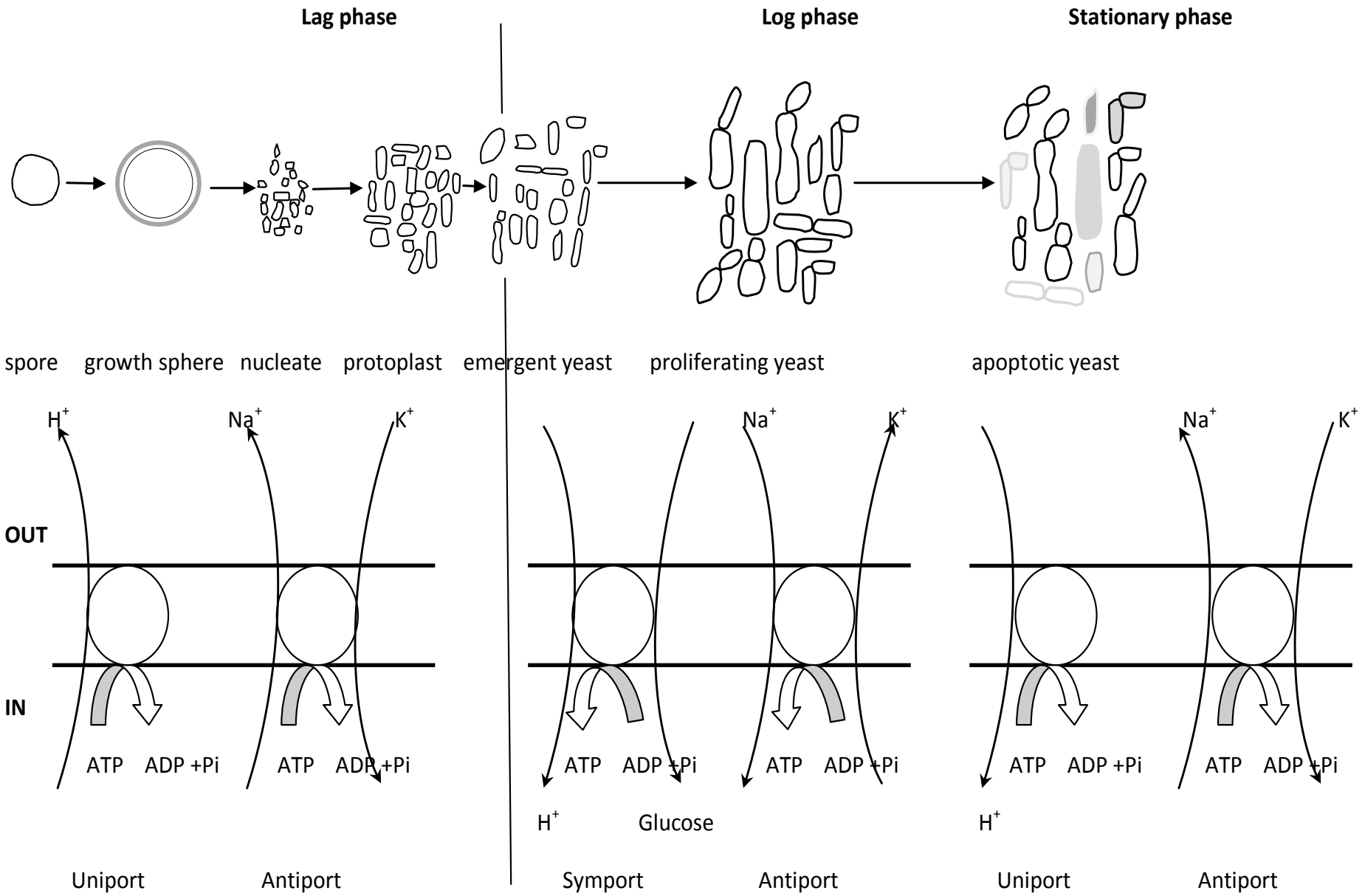

Figure 59. Scheme illustrating possible modes of behaviour of transport processes in the growth phases during sporangiospore-yeast transformation of *M. circinelloides* Tieghem, cultivated in buffered glucose-substrated multiionic broth for 120h at pH 4.5, temp.20^{o}C, ambient. The concentrations of K^{+} and Na^{+} were 1.0 and 0.1g/l, respectively.

Table 1. Variation in the ratio of intracellular ion concentration during sporangiospore-yeast transformation of *M. circinelloides* Tieghem cultivated in 1.0 g/l K^+- mediated and 0.05 to 0.20 g/l Na^+-modulated multiionic broths. Determinations were done at 24 h intervals.

Bulk medium concentration (g/L)		Intracellular medium ratio	
K^+	Na^+	Na^+	K^+
		3.86	3.30
		2.30	3.27
	0.05	2.80	1.40
		3.57	2.78
		4.70	3.77
		3.03	2.20
		2.10	2.80
	0.10	6.00	2.01
		2.40	2.20
		2.32	2.70
1.00			
		6.65	2.47
		2.37	3.03
	0.15	3.67	1.72
		4.58	4.33
		2.22	2.77
		2.40	2.25
		1.52	1.68
	0.20	2.50	1.48
		1.72	2.40
		6.00	3.40

in the intracellular medium during the lag phase in the transforming-cooperative system. Since the commencing morphology was distinct, the lag phase encompassed phenotypic as well as metabolic adjustment prior to exponential growth. Phenotypic adjustment sequentially included growth sphere formation, cytosolic nucleation, protoplast formation and the yeast form development. Protoplast formation was critical to subsequent transition to the yeast form. Carbonell et al., (1973) have shown the ability of the yeast form of *Histoplasma capsulatum* to regenerate from its protoplast. But, our study showed that chemical potential was significant to nucleation. The higher the value of *m,* the farther away from cytosolic nucleation was the cooperative system. This became more pronounced in the non-cooperative systems where thallic growth was predominant.

Our model cooperative system had the highest specific growth rate during the exponential growth phase, a fact reflected in the Na^+ influx rate. This meant that in this study, the high Na^+ influx rate was also a measure of the specific growth rate, and it signified very high rate of biosynthetic activities, including intracellular communication, metabolic differentiation and cellular division. Table 1 shows that the intracellular concentration of Na^+:K^+ increased tremendously, by a ratio of 6.0:2.01. The ATP/ADP ratio would perhaps increase accordingly. At optimum level of ATP production, biosynthetic activities could peak, and thereafter, there would be no further need for it. Thus, the ATP level would fall. This was inferred from the drastic fall in the Na^+/K^+ ratio, which almost came to par, 2.4:2.2, at the stationary phase of growth. Apoptosis however heightened with the Na^+/K^+ ratio further, decreasing (2.3:2.7), as the balance of ions favored more K^+ influx into the intracellular medium (Table 1). This study shows that ionic (Na^+/K^+) balance could be important in the programmed cell death.

Another form of cell death was observed in this study. This was cell necrosis. This involved the rupturing of cells of septate hyphae or bursting of conidia where after, the cytosolic contents were spilled into the environment. The corpse thereafter shriveled and conidia shell collapsed. The shrunk filaments were seen as fragile hyphae. These events were also seen in the case of *D. diastaticus* strain C12, IMI W5132A (Omoifo, 1997). One interesting fact was that in the determinate thallic growth, phenotypic change was inherent: nucleation occurred in each cell; these transited to protoplasts with consequent volume changes and the building up of internal pressure, which possibly then caused cell rupture. On release, the protoplasts commenced independent growth and thus

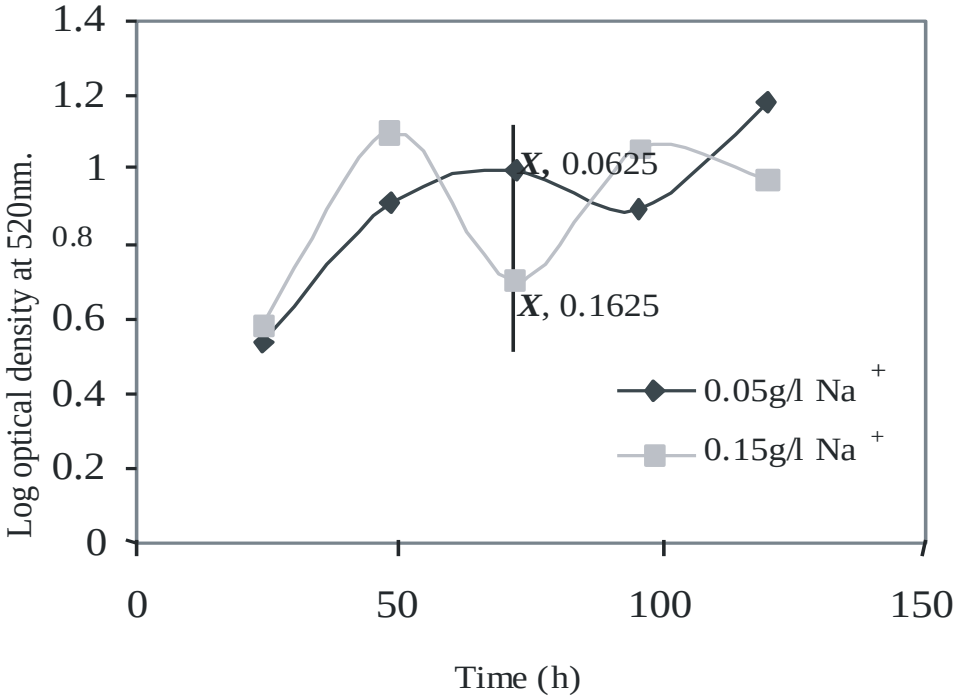

Figure 60. Non-cooperative growth curves for sporangiospore-yeast influx rates at exponential growth phases, of *M. circinelloides* in broth charged with 1.0 g/l K^+; x, Na^+ influx rate.

transited to the yeast morphology, which then budded off daughter cells. This pattern description was seen at the 1 g/l K^+-0.05 g/l Na^+ charge where the first peak represented determinate thallic growth optimum, followed by a fall and subsequent rise in optical density measurement as a result of yeast growth. Similar phenomenon was clearly demonstrated at 1.0 g/l K^+-0.15 g/l Na^+ charge where the 2nd growth optimum was mainly contributed by the protoplasts. Although yeast cells evolved, they were scantier than the aforementioned ionic charge-system and, hence, the fall in optical density reading (Figure 60). In our categorization, these two growth patterns represented the non-cooperative forms of sporangiospore-yeast transformation of *M. circinelloides* Tieghem. It was suggested that the intracellular ionic ratios for the non-cooperative system differed from those of the highly functional cooperative system (Table 1) because of (a), the barrier posed by the high chemical potential to nucleation and (b), the occurrence of the phenomenon of cell necrosis.

Reference has been made to the occurrence of thallic growth even in the cooperative system. This was not new. *D. ubiazae* strain C16, IMI W5132C exhibited mainly thallic growth, but no yeast cell (Omoifo, 2005) in similar conditions that we have classified as cooperative, but the filament of *M. circinelloides* Tieghem was outstanding. While those of *D. ubiazae* strain C16, IMI W5132C were brown septate hyphae with conidioygenously produced catenate conidia and hyaline coenocytic hyphae with sporogenously produced catenate conidia, the olive-green septate hyphae of *M. circinelloides* Tieghem produced conidiophores which terminated in an enlarged subglobose vesicle from which originated chains of globose to subglobose conidia; each chain could be made up of up to 25 spores. On the other hand, morphological expression of *D. diastaticus* strain C12, IMI W5132A in submerged cultures included septate hyphae from which sporophores emerged, bearing sporangia, albeit atrophied, only when the non-buffered glucose-substrate multiionic broth pH 3.5, was supplemented with choline chloride, nicotinic acid or riboflavin (Omoifo, 1997). When the inoculum was sporangiospores of *D. pleomorphis* strain C13, IMI W5132B, terminal budding ellipsoidal yeast cells, but not thallic expression (Omoifo, 1996b), were induced in similar cooperative system that induced mainly obpyriform yeast cells along with scanty septate hyphae from which emanated conidiophores bearing vesicular conidial head group in the present study.

These results led to the conclusion that the *Dimorphomyces* strains were distinct from *M. circinelloides* Tieghem. The differences could be generic.

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Contributors: The concept, design, analysis and write-up were by COO; BIA participated physically in the study.

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