

Full Length Research Paper

Effect of K⁺, Na⁺ and uracil on sporangiospore-yeast transformation of *Mucor circinelloides* Tieghe

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Accepted 16 February, 2006

Potassium is a key constituent element that functions in intracellular metabolism of eukaryotes. This study demonstrates potassium is absolutely necessary for the formation of *Mucor circinelloides* protoplasts, the cross over transient form in the sequential transformation of sporangiospores to yeast cells. We also show that Na⁺ contributes significantly to the induction of yeast form from protoplast in synthetic broth and this is time related. Two distinct anamorphic patterns were induced, initialized by thallic growth and growth sphere wall lyses. Thallic growth was determinate with subtype's production of conidia after growth cessation as in vesicular head group conidial form or enterothallic conidia and catenulate formation of conidia on same plane with short germ tube represented by holothallic conidia, as well as holoblastic conidia. The second growth pattern was proliferate, with yeast cell giving rise to daughter buds. Biomass profile exhibited 2-phases common in Na⁺ supplemented broths with determinate thallic growth contributing proportionally to the first peak. On thallic cell wall rupture, released cytosolic units progressively convert to protoplasts and subsequently to yeast cells. Uracil supplementation eliminated determinate thallic anamorphs with morphological expression occurring as ovoid to long ovoidal, ellipsoidal and cylindrical yeast cells, which were polar, bipolar or multilateral budding. Thin tree-like branched yeasts were scanty at 100 mM uracil supplementation. The results offer new insights into the phenomenon of fungal morphogenesis. It attributed specific effects to K⁺, Na⁺ and uracil as key factors to which *Mucor circinelloides* respond, phenotypically.

Key words: *Mucor circinelloides*, biomass, induced morphology, thallic subtypes, protoplasts, yeast cells.

INTRODUCTION

Literature in the last century is replete with studies on the effect of environmental factors on fungal dimorphism. With specific reference to *Mucor* species, environmental factors that have been found to effect mycelia-yeast inter-conversion include carbon dioxide pressure (Bartnicki-Garcia and Nickerson, 1961, 1962), hexose concentration (Bartnicki-Garcia 1968, Bartnicki-Garcia and Nickerson, 1962), inoculum size (Elmer and Nickerson, 1970), systemic fungicide (Fisher, 1977), mitochondrial inhibitor (Friedenthal et al., 1974; Fisher, 1977), respiratory inhibitor (Clark-Walker, 1973), exogenously applied dibutyryl cAMP (Larson and Sypherd, 1974; Orłowski, 1980; Paveto et al., 1975; Paznokas and Sypherd, 1975), oxygen-free nitrogen (Mooney and Sypherd, 1976), fatty acid inhibitor (Ito et al., 1982) and glucose repression (Paznokas and Sypherd, 1975; Rogers et al., 1975).

A mucoraceous strain with culture collection Specimen number, W5132B (IMI, 1996) was isolated and given the

tentative form-genus, *Dimorphomyces* (Omoifo, 1996a). It proved to be dimorphic. A scheme for the transformation of its sporangiospores to terminal budding yeast cells has been presented (Omoifo, 1996b). In synthetic batch culture a spore converted to growth sphere, then cytosolic units or nucleates and subsequently, yeast cells. These became terminal budding. In a subsequent publication, it was shown that the formation of protoplast was critical to the final development of the yeast morphology (Omoifo, 2003). Logical theorizing deposed that it formed the base architecture on which the yeast cell wall was built (Omoifo, 2003).

It was proposed that the protoplast afforded ionic communication between intracellular medium and the bulk medium (Omoifo, 2003). This is akin to the inner mitochondrial membrane of bacterial cell, which allows stoichiometric H⁺- substrate symport (West and Mitchell, 1972, 1973) in a vector ally directed chemiosmotic process (Mitchell, 1961). Similar processes have been

found to occur in other eukaryotes, including the yeast, *Saccharomyces cerevisiae* (Seaston et al., 1973), *Metchnikowia reukaufii* (Alderman and Hofer, 1981), and the fungus, *Neurospora crassa* (Slayman and Slayman, 1974).

In such vectorally directed process, extrusion of protons from the intracellular medium would lead to a further decrease of pH in an acidic bulk medium. This was demonstrated during the first phase of sporangiospore-yeast transformation of *D. pleomorphis* strain C13, IMI W5132B where the transient morphologies were observed to be growth spheres, nucleates and protoplasts (Omoifo, 1996b). Since proton movement is linked with K^+ , as yeasts exchange K^+ for cellular protons (Conway and O'Malley, 1946; Pena, 1975), it was thought that K^+ would play a role in intracellular activities mediating the transformation of sporangiospores to yeast cells. In support of this assertion, it has been found that the proton release intensity varied with concentration of K^+ in the bulk medium inoculated with sporangiospores of *M. circinelloides* (Omoifo, 2005). Perhaps as proton was being released to the bulk medium, and as such internal pH increased, there was simultaneous influx of K^+ into the intracellular medium. Ryan and Ryan (1972) showed that K^+ movement is dependent on intracellular pH. But while the proton release intensity was optimum at 1.0 g/l, it was at par with the control experiment in the study referred (Omoifo, 2005). However, microscopic examination did not reveal protoplasts in the control study. It was concluded that K^+ was of necessity for protoplast formation, and subsequently yeast form induction. It is well known that K^+ is exchanged for Na^+ (Tonomura, 1986). Although the multiionic broths that induced terminal budding yeast cells from sporangiospores had Na^+ supplementation (Omoifo, 1996b, 1997, 2003), the role played in sporangiospore-to-yeast transformation has not been examined.

Studies in our laboratory have shown that when the medium of cultivation of the form-genus, *D. pleomorphis*, strain C13, IMI W5132B was incorporated with growth promoting factors, including nicotinic acid, riboflavin, thiamine.HCl, biotin, chlorine chloride, uracil, thymine, calcium pantothenate, inositol, ascorbic acid and yeast extract, multipolar budding yeastlike cells, conidia, cytosolic nucleates and mycelia fragments were induced, in addition to terminal budding yeast cells, which were preponderant (Omoifo, 2005). On the other hand, a related mucoraceous strain, *D. diastaticus* strain C12, IMI, W5132A required the pyrimidine base, uracil, to convert to terminal budding yeast morphology (Omoifo, 1997). Perhaps similar effect would be executed in the case of *M. circinelloides*.

The objectives of this study were to determine the influence of multiionic broth incorporated with (a) single valence ions K^+ , Na^+ and (b) a pyrimidine base, uracil, on the transformation of sporangiospores to yeast cells.

MATERIALS AND METHODS

Fungal strain and maintenance

The organism, *M. circinelloides* Tieghe, used in this study was first isolated from decayed fruit of soursop, *Annona muricata* L., obtained from the floor bed of the tree. It has been used in earlier studies (Omoifo, 2005). It was maintained as glucose-yeast extract-peptone (GYP: 10: 03: 5 g/l) solid cultures where it exhibited filamentous growth habit. A fresh culture was prepared after seven days.

Inoculum preparation for growth studies

Inoculum was obtained by pouring sterile deionized distilled water over aerobic growth and a sterile glass rod gently passed over the surface so as to dislodge the spores. The suspension was poured into centrifuge tubes and spores washed by centrifuging at 5000 rpm for 7 min at 25°C in an MSE 18 centrifuge. The wash was decanted, sediment re-suspended and further washed with two changes of sterile deionized distilled water. Spore count was taken with Neubauer haemocytometer (BSS No. 784, Hawksley, London vol. 1/4000) and was adjusted to 1 million spores per ml in sterile deionized distilled water, with the aid of a tally counter.

Reagents and culture media

All reagents for the culture medium were obtained from BDH Laboratory supplies (Poole, UK). Media were prepared per litre: glucose, 10.0 g; $(NH_4)_2SO_4$, 5.0 g; $MgSO_4 \cdot 2H_2O$, 2.0 g; $FeSO_4 \cdot 7H_2O$, 0.10 g; $MnCl_2$, 0.065 g; $CuSO_4 \cdot 7H_2O$, 0.06 g; $ZnSO_4 \cdot 5H_2O$, 0.06 g; NaCl, 0.10 g. Media were prepared in 5000 ml beakers. Weights of buffer components 0.2 M Na_2HPO_4 , 0.1 M citrate were obtained using H54AR mettler balance and added to the beaker. A preliminary experiment was conducted using various levels of K^+ and thereafter three levels, which were found outstanding to yeast induction were chosen and varied with four levels of Na^+ for this study. Since the effects of K^+ and Na^+ were to be tested, each of duplicate broth flasks was incorporated with the various concentrations of KH_2PO_4 : 0.90, 1.00, and 1.10 and of NaCl: 0.05, 0.10, 0.15, and 0.20 g/l. The effect of uracil was also tested and each of duplicate broth flask was incorporated with the various concentrations 0.0, 20.0, 50.0, 100.0, 150.0, 200.0 mM. The pH was adjusted to 4.5 with 2 N NaOH or 1 N HCl, using a Cole-Parmer pH Tester model 59000, in the 5000 ml beakers before dispensing or in an 80 ml of broth in each of duplicate 250 ml Erlenmeyer flasks for each test. The solution in each flask was made up to 100 ml with glass distilled deionized water and sterilized at 121°C for 15 min.

Inoculation, growth conditions and sample collection

A 1 ml of spore suspension was drawn and inoculated into each broth flask using a 0.5 ml rubber suctioned pipette in a laminar flow chamber, model CRC, HB-60-180. The inoculum flask was shaken at each operation so as to keep the spores in suspension. Each culture flask was then shaken for 30 s and thereafter incubated at 20°C in a preset cooled Gallenkamp incubator. At 24 h interval the culture flasks were brought to the inoculating chamber. The flasks were shaken; 10 ml of broth was withdrawn and deposited into factory-sterilized plastic sample tubes, pre-labeled for each experiment. The culture flasks were returned for further incubation and samples kept at -18°C until analysis.

Table 1. Analysis of variance (ANOVA) of growth data of *M. circinelloides* cultivated in buffered synthetic broths with different levels of K⁺ and Na⁺ incorporation.

Source of variation	d. f.	Sum of squares	F-values
Time	4	3.45264	<0.001*
K ⁺ - level	2	0.11181	0.471
Na ⁺ - level	3	0.74751	0.023*
Time x K ⁺	8	0.23804	0.913
Time x Na ⁺	12	0.06363	0.583
K ⁺ x Na ⁺	6	0.60443	0.556
Time x K ⁺ x Na ⁺	24	0.05596	0.764
Residual	60	0.07330	
Total	119		

* F-values significant at p<0.05

Table 2. Homogeneous subsets of mean biomass estimates obtained during sporangiospore-yeast transformation of *M. circinelloides* cultivated in Na⁺- K⁺ incorporated synthetic broth for 120 h at pH 4.5, temp 20°C.

Treatment,	Mean
Subset 1	
0.05 Na ⁺ (g/l)	0.915
0.10 Na ⁺ (g/l)	0.977
Subset 2	
0.15 Na ⁺ (g/l)	0.812
0.20 Na ⁺ (g/l)	0.779
Means were separated using l.s.d., p < 0.05, 0.1396	

Biomass determination

Culture broth samples were thawed up to room temperature before biomass determination. This was done by measurement of optical density at 520 nm. This wavelength was chosen because ordinarily FeSO₄ impacted greenish coloration on culture broth. Absorbance was determined with a Grating spectrophotometer CE 303 (Cecil Instruments, Cambridge) and with a Camspec M105 spectrophotometer (Cambridge, UK), which was then available during uracil-incorporated growth studies.

Statistics

Results were subjected to a 2-way analysis of variance (ANOVA) test for the single factor, or a split-plot format for combined factors and considered significant if p < 0.05 and comparison between means was performed using the Genstat 5 package.

RESULTS AND DISCUSSIONS

Considerable differences were observed in the growth of the microorganism in the presence of treatment levels of K⁺ and Na⁺ ions. Analysis of variance showed that of the

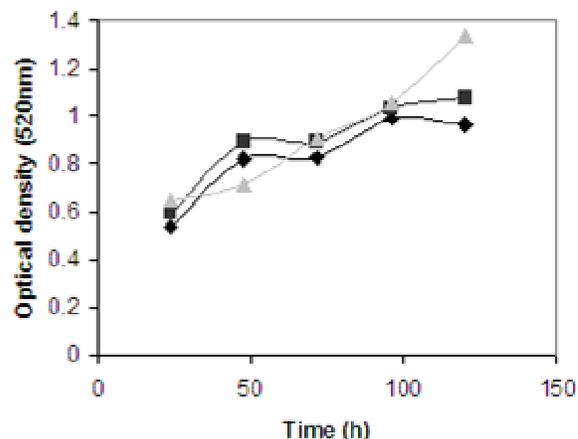


Figure 1. Effect of K⁺ on biomass accumulation during sporangiospore-yeast transformation of *M. circinelloides* cultivated in buffered synthetic broth for 120 h at pH 4.5, temp 20°C, ambient. (◆), 0.90g/l; (■), 1.0g/l; (▲), 1.10g/l.

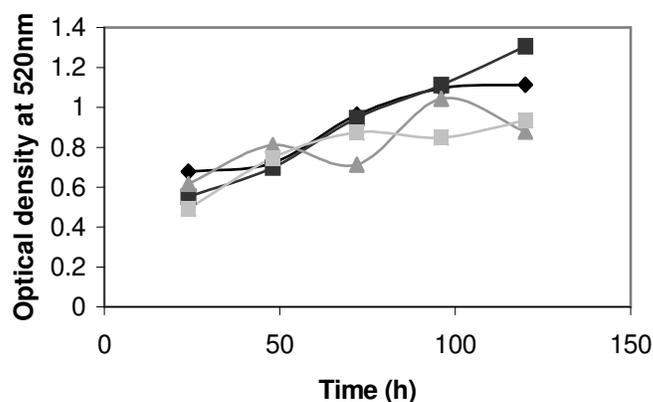


Figure 2. Effect of Na⁺ during sporangiospore-yeast transformation of *M. circinelloides* cultivated in buffered synthetic broth for 120h at pH 4.5, temp 20°C, ambient. (◆), 0.05g/l; (■), 0.10g/l; (▲), 0.15g/l; (▼), 0.20g/l Na⁺.

main factors only time at p<0.001 but Na⁺ at p<0.05 contributed effectively to growth (Table 1). Separating biomass mean values by the lsd (p<0.05) 0.1396, gave two homogenous subsets for the Na⁺ levels, the highest being at 0.10 g/l Na⁺, which shared the same significance with 0.05 g/l Na⁺ as distinct from subset 2 (Table 2).

There was no significant interaction between K⁺-level and time. However, simple response of biomass profile showed a higher plot at late growth phase for the 1.10g /l K⁺-level, in comparison to the other K⁺-levels (Figure 1).

Although there was noticeable difference in the shape of Na⁺-time curve at the 0.15 g/l Na⁺-level, being more cryptic and resulting in two optima, the direction of growth was similar to the other levels (Figure 2). However after 120 h, 0.10 g/l Na⁺-level had the higher slope. On the

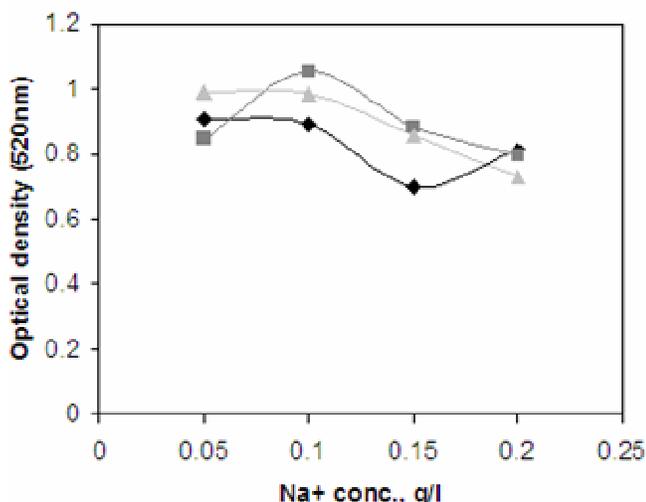


Figure 3. Effect of interaction between K^+ and Na^+ on the growth of *M. circinelloides* cultivated in buffered synthetic broth for 120h at pH4.5, temp 20°C, ambient. (◆), 0.90g/l; (■), 1.0g/l K^+ ; (▲), 1.10g/l K^+ .

Na^+ - K^+ interaction, which was not significant (Table 1), profiling showed that biomass production was maximal at 1.0 g/l K^+ : 0.10 g/l Na^+ treatment (Figure 3).

Since the physiology of this organism in synthetic broth has not been fully established, biomass determination alone is only a partial representation of the growth habit. This makes microscopic examination imperative. Such examination showed growth classified into two distinct anamorphic habits: determinate thallic and discrete proliferating patterns. Determinate anamorph consisted of (a) production of asexual spores after growth had ceased, as we find in (i) the septate thallic growth with vesicular head group with radiating conidial chains and (ii) septate and non-septate thallic growths, which thereafter produced enterothallic conidia, (b) production of asexual spores on the same planar axis as growth occurred with or without short germ tube, as we find in holothallic and holoblastic conidia. The proliferating growth habit involved conversion of sporangiospores to growth spheres from which derived cytosolic units released as granular particles, enlarging to protoplasts and subsequently, yeast form development; the yeast cells were terminal budding.

The 1.0 g/l K^+ : 0.10 g/l Na^+ treatment, which had the optimum biomass accumulation, also induced the greater proportion of terminal budding yeast cells. Other morphologies induced in this ions combination included the aforementioned thallic subtypes, although highly reduced presence in comparison with the other treatments. Yeast cells also induced in the other treatments including 0.9 g/l K^+ with 0.05 - 0.20 g/l Na^+ , 1.0 g/l K^+ with 0.05 - 0.20 g/l Na^+ , and 1.10 g/l K^+ with

0.20 g/l Na^+ , although the proportion in each case was smaller. Observation also showed that the proportion of yeast cells increased with increase Na^+ supplementation up till the 0.10 g/l Na^+ level.

Biomass profiles of growth were shown in Figure 4. Since the 1.0 g/l K^+ with 0.10 g/l Na^+ treatment induced the greater proportion of terminal budding yeasts, it could well form the reference point. Biomass profile at this treatment exhibited 2 peaks: after 48 h and 96 h. The 0.9 g/l K^+ challenge at the 0.10 g/l Na^+ charge mildly described similar pattern but profile at 1.10 g/l K^+ at this Na^+ charge was barely lineal. At the lower Na^+ charge, biomass profile at the 1.0 g/l K^+ also approximated 2 peaks, the first shifting to 72 h following spore inoculation, but was still on the increase after 120 h. There was a one-peak manifestation at 0.9 g/l K^+ conc. A 2-peak display also occurred at the 1.0 g/l K^+ - 0.15 g/l Na^+ treatment and it was roughly so at the other two levels. Profiles at the 0.20 g/l Na^+ ionic charge were 2-peaked, except the 1.0 g/l K^+ challenge, which was sigmoid. But the first peak at the 1.10 g/l K^+ challenge took more time to accomplish.

Of particular note is the double peak manifestation at the 1.0 g/l K^+ challenge at all Na^+ charges, except 0.20 g/l; yeast cells also predominated such high level K^+ broths. Since protoplast is the key intermediate form for yeast morphology development (Omoifo, 2003), the predominance of yeast cells further supports earlier findings that high K^+ ionic challenge was necessary for the transient protoplast formation (Omoifo, 2005). Although statistic did not indicate any interaction between K^+ and Na^+ , microscopic examination showed that proliferating yeast cells were more preponderant when the high K^+ challenge was charged with 0.10 g/l Na^+ . Perhaps, it could be inferred that a measure of cooperativity existed between the two ionic species at the intracellular levels. Such inference was derived from the fact that ionic challenges, primarily involving K^+ and Na^+ , generate membrane potentials that trigger transduction of mitogenic signals for consequent mitotic activity whereby somatic cells undergo active proliferation (Cone, 1985). Similar effect probably occurred in the previous studies of Omoifo (1996b, 1997), since the media of growth were incorporated with similar level of the individual ionic species.

However, the present study differed from that of Omoifo (1996b), which exhibited only proliferate yeast as the terminal morphology. In the determinate growth habit as observed in the present study, spores converted to growth spheres, then thallic, which produced conidia after cessation of growth, or first produced germ tube and subsequently catenulate conidia. This, in addition to the transient forms of the sequential sporangiospore-yeast transformation (SSYT) process (Omoifo, 2003), possibly caused the increase in biomass leading to the first recorded peak. Since the SSYT process involved generation of specific transient morphologies from

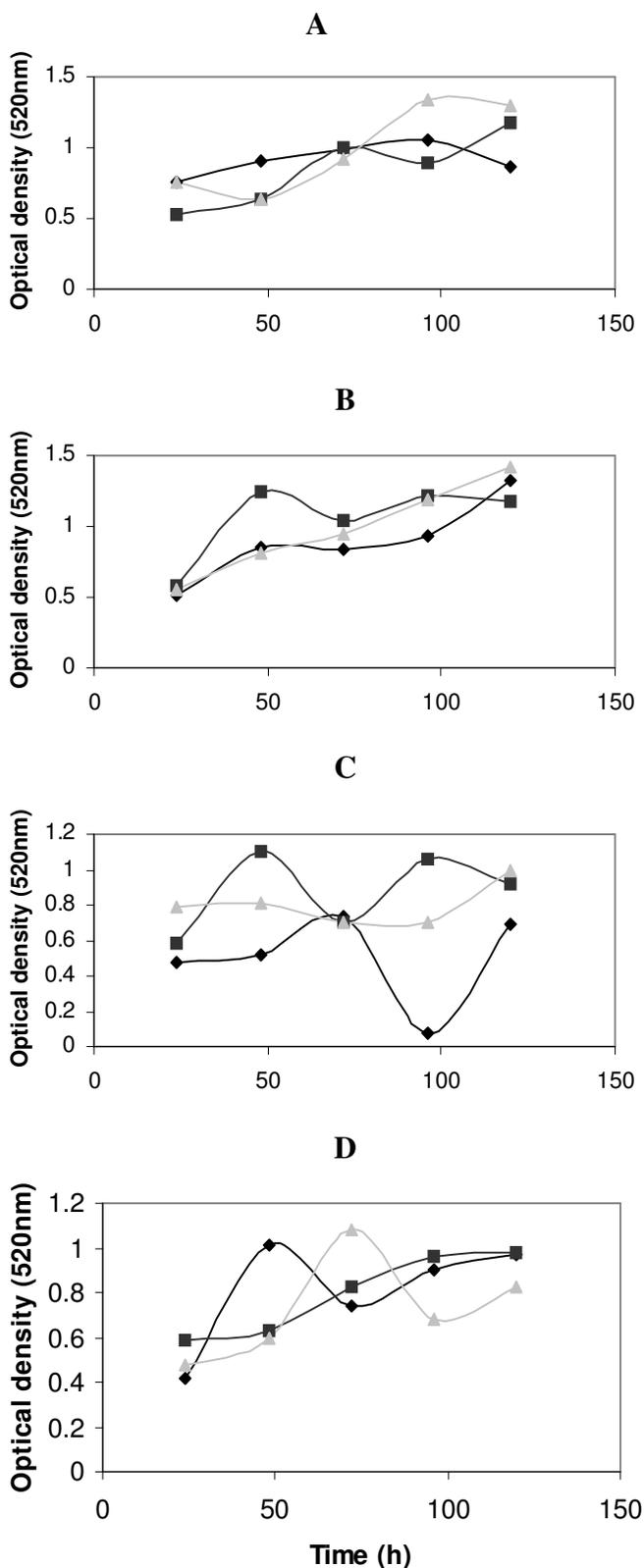


Figure 4. Biomass profiles of *M. circinelloides* cultivated in buffered synthetic broth with K⁺- and Na⁺- supplementations for 120h at pH4.5, temp 20°C, ambient. A, 0.05g/l; B, 0.10g/l; C, 0.15g/l; D, 0.20g/l. Na⁺. (◆), 0.90g/l; (■), 1.0g/l; (▲), 1.10g/l K⁺.

Table 3. Analysis of variance of growth data of *M. circinelloides* cultivated in buffered synthetic broths with different levels of uracil incorporation.

Source of variation	d. f.	Sum of squares	F-value
Uracil level	5	0.94690	0.053
Time	4	1.20674	0.011*
Uracil level x time	20	2.61782	0.087
Residual	30	2.27950	
Total	59	7.050960	

* F-value significant at p<0.05.

sporangiospores, from which yeast cells induced (Omoifo, 1996b, 1997, 2003), observation showed that this later morphology was the primary contributory to the second peak of the growth profiles of *M. circinelloides* in this study. But ruptured thallic-cell released cytosolic nucleates which converted to protoplasts from which yeast cells developed, was also contributory. The non-occurrence of sigmoid growth habit during sporangio-spore-yeast transformation in this study is not new, as this has been reported in several studies (Omoifo, 1996b, 1997, 2003). It could be attributed to the structural modifications involving several distinct transient phenotypic expressions associated with the transformation process. The contributory role of K⁺ to protoplast formation, then Na⁺ and time to yeast morphology induction was outstanding.

Analysis of variance of growth data obtained in the culture broths with uracil supplementations showed that time, as main factor, made significant contribution to growth (Table 3). But neither uracil levels with 5 d.f. nor its interaction with time made such an impact. Means separation, lsd at p<0.05, 0.2518 gave two separate homogenous subsets: biomass being optimum at 0.0 (0.751 nm) and 100.0 mM (0.594 nm) of subset 1, while it was least at 200.0 mM (0.396 nm) uracil supplementation but this had the same significant effect with 20.0 (0.399 nm), 50.0 (0.452 nm) and 150.0 mM (0.44 8nm) broths of subset 2. Biomass profiles were shown in Figure 5. Even though yield in control test had the same significance with 100.0 mM, it was only the latter that approximated determinable growth pattern consisting of a lag phase, exponential phase, stationary phase and a death phase. Profiles of treatments in subset 2 did not approach this habit.

Variability in biomass was greater in the control test and it was also reflected in morphological forms. Microscopic examination showed varying sizes and shapes of induced yeast cells that included ovate, pyriform, obpyriform, ovoidal, oblong, ellipsoidal, cylindrical, filiform and elongate types, which also formed fragile tree-like branching yeasts, occurring in clusters and apparent meshwork. Terminal or lateral blastopores

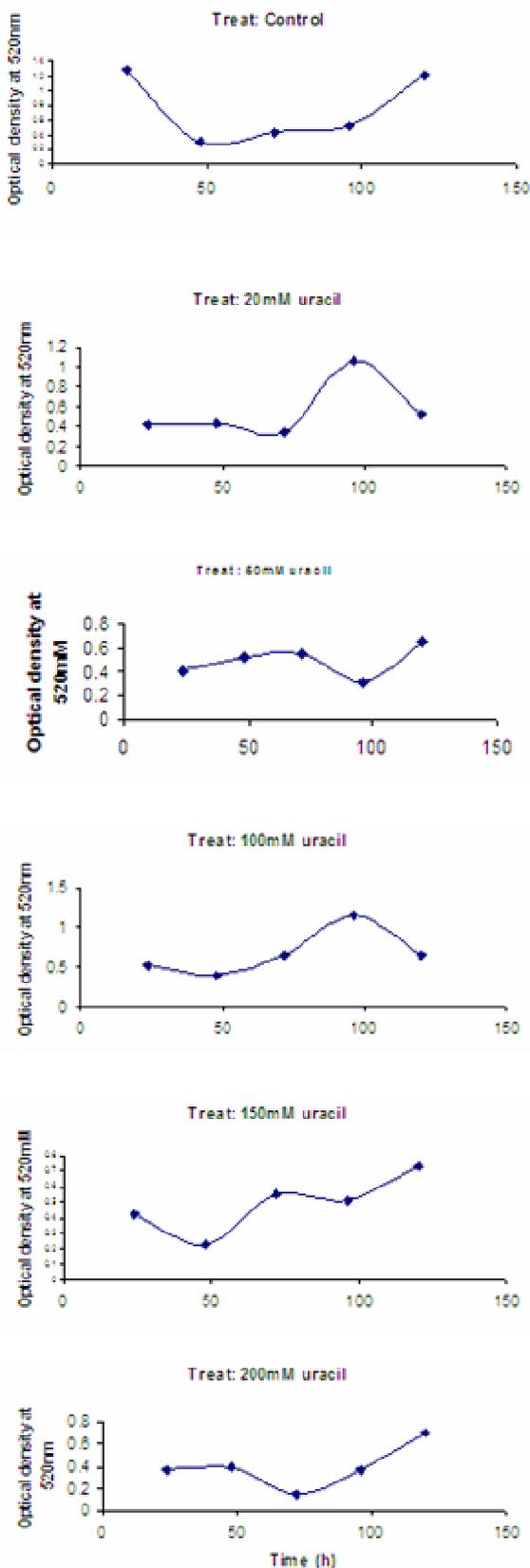


Figure 5. Biomass profiles of *M. circinelloides* cultivated in synthetic broth incorporated with varying levels of uracil.

on these thin-celled tree-like branching yeasts could be globose, subglobose, ovoid, long ovoid, ellipsoid or teardrop-like. But individual yeast cells were terminal budding. Cytosolic nucleates occurred clumped or dispersed. With 20 mM uracil supplementation, there was reduction in the tree-like clusters; nucleates dispersed or in clumps also assumed primordial yeast forms; the oblong and ovoid yeast cells assumed larger sizes and some cells assumed fine egg shape. Ovoid and long-ovoid yeast cells were more numerous at 50 mM-supplementation and could occur as singles, doubles, short chains or in clusters or bud multilaterally, while the single elongate types and tree-like branched forms diminished but could release single, terminal, or lateral blastopores, which could be ovoid to long ovoidal, ellipsoidal or cylindrical. Cytosolic nucleates, dispersed or in clumps, and primordial yeast forms occurred. There were no nucleates at the 100 mM supplementation. Primordial yeast cells occurred but optimum-size yeast cells were ovoidal, ellipsoidal, elongate, and could be terminal, bipolar or multilateral budding; the tree-like branched yeasts were scantier. At 150 mM-level, yeast cells were ovoidal, ellipsoidal and multilateral budding; occurred as singles, doubles or in short chains. All the aforementioned types of cells were represented at the 200 mM uracil supplementation but primordial yeast cells were numerous.

This study demonstrated the effects of major elements like K^+ and Na^+ on the growth of *M. circinelloides*. Since previous work shows that the establishment of a transmembrane-pH-gradient permitted the sequential sporangiospore-yeast transformation process (Omoifo, 2003, 2005) and further appropriated a Mitchellean proton pump mechanism (Omoifo, 2005), then such transport processes involving K^+ and Na^+ were deemed to have occurred in the present study. Studies have shown that a continuous antiport movement of K^+ and Na^+ occurred between the intracellular and bulk media during the transformation process of sporangiospores to yeast cell of *M. circinelloides* (Omoifo, 2005). That yeast cells were induced in the control tests in the present study, further gives credence to previous claim on the aforementioned processes. However, the occurrence of apparent meshwork of elongate yeast cells probably account for the irregular growth pattern obtained at this level, as such growth could have given rise to the high biomass at the early stages of growth, in contrast to discrete yeast forms at the higher levels of uracil. While thallic cell contents could be modified into cytosolic particles, thereafter released as individual units, and, or protoplasts on cell wall rupture in monovalent ion supplemented cultures, determinate thalli were absent with uracil-supplementation. Rather, the presence of apparent meshwork of tree-like branched yeast cells diminished as uracil concentration increased and yeast form became more streamlined, reaching an utmost representation at 100 mM level.

Contrast was also made with *D. diastaticus* strain C12, IMI W5132A, which induced only, nucleates, conidia, and septate mycelia in similarly glucose-substrate media (Omoifo, 1997). When the medium was supplemented with 100.0 mg/l uracil, terminal budding yeast cells were additionally induced (Omoifo, 1997, 2003). Explanation for this was based on salvage pathway generation of UMP from which formed UTP catalyzed by nucleotide diphosphokinase (Omoifo, 2003). In the presence of CTP synthetase, CTP could be generated from UTP. This nucleotide could be used for the formation of activated intermediates, like CDP-diacylglycerol, CDP-ethanolamine, CDP-phosphatidylserine, and CDP-choline, which are involved in phospholipid metabolism in eukaryotic cells. Phospholipids give definition to cellular structures as well as participate in intracellular processes in an integrated manner with other aspects of cell physiology (Carman and Henry, 1989; Carman and Kersting, 2003). Since yeast cells were co-induced with septate mycelia in the study referred (Omoifo, 1997), conditions occurred that permitted the two developmental pathways. The suggested explanation on reactive oxygen intermediates (Omoifo, 2003) could be attributable.

In control broths for uracil-fed study, absence of determinate thalli was perhaps due to de novo synthesis of uracil and consequently nucleotides from which derived energy-rich phospholipid intermediates which would lead to synthesis of well partitioned structure integrated and phospholipid signal mediator molecules, including phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylcholine, as occurs in *Saccharomyces cerevisiae* (Carman and Henry, 1989; Paltauf et al., 1992; Sreenivas et al., 2001). Therefore, uracil supplementation advanced the commencement point in the molecular and biochemical progress to the yeast morphology, and hence a further streamlining and regularity of induced yeast forms, which was optimum at 100.0 mM uracil. There from, was the enhancement of integrated molecular activity leading to mitotic division with the inherent terminally budded daughter cells, as in *S. cerevisiae*. Similar conclusion was reached for the transforming uracil-auxotroph, *D. diastaticus* strain C12, IMI W5132A (Omoifo, 2003).

The occurrence of apparent meshwork of tree-like branched yeast cells in the control tests, and with diminishing presence in uracil supplemented broths, presently could not be accounted for. Perhaps, the exhibition of the oxygen effect (Omoifo, 2003), whereby non-enzymes reactions in an anaerobic growth environment led to the generation of reactive oxygen intermediates, thus stimulating aerobic processes (Michelson, 1978), the effect of which appeared limited in scope, possibly as a reflection of ability of uracil-induced reactions to overcome oxidative stress, could be considered.

ACKNOWLEDGEMENTS

This work was done in the Pathology Division of the Nigerian Institute for Oil Palm Research Benin City. To the Director, Dr. U. Omoti, I express gratitude. I also thank the Head of the Pathology Division, Dr. (Mrs.) C. Airede and her staff for cooperation. The advice of Dr. C. E. Ikuenobe on statistical analysis is gratefully acknowledged.

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