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Full Length Research Paper

Effect of myoinositol and zinc on sporangiospore-yeast transformation of *Mucor circinelloides* Tieghe

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Environmental factors influence mould-yeast interconversion. Such factors may trigger changes at the cellular level. Yeastlike cells induced from sporangiospores of *Mucor* species consist of central globose mothercell, which produce multiple buds by blastic action. This study examines the effect of synthetic broth on sporangiospores of *M. circinelloides*. Sporangiospores of *M. circinelloides* converted to determinate thallic subtypes including enterothallic- holothallic- and holoblastic conidia, as well as terminal budding yeast cells in synthetic broth treatments with zinc and myoinositol (myo) supplementations. In a study monitored at 24 h intervals, profiling gave 2-phase expression in some treatments and this was influenced by the presence of thallic forms. However, sigmoid pattern was obtained in control broth as well as in 0.25 mM Zn^{2+} and 1.0 – 3.0 mM myo treatments. Such treatments enhanced growth by 32.95, 65.07 and 63.82%, respectively, over control mean growth. Proliferating yeast cells induced from sporangiospores of *M. circinelloides* transformed through several transient morphologies in a sequential manner, protoplast being the cross over form. Zinc and myo supplementation enhanced yeast induction. It was strongly suggested that they acted as second messengers in signal transduction and consequent yeast induction. Whereas thallic subtypes were present, subsequent transformation of their cytosolic contents, following thallic cell wall rupture, followed the same pattern of lateral morphogenetic transformation.

Key words: *Mucor circinelloides*, sporangiospores, synthetic broth, zinc, myoinositol, induced morphology.

INTRODUCTION

Members of the Mucorales have either monomorphic or dimorphic growth habit (Ruiz-Heirera, 1985). Dimorphic *Mucor* species naturally produce coenocytic tubular filaments, which give rise to sporangiophores that terminate with columellae enclosed within sporangia. Under anaerobic conditions, *Mucor* species assume isotropic growth habit, producing daughter buds from different loci by blastic action. Such multipolar budding yeastlike growth habit has been recorded for *Mucor circinelloides, M. racemosus* and *M. rouxii* (Bartnicki-Garcia, 1968; Bartnicki-Garcia and Nickerson, 1962 a,b; Friedenthal et al., 1974; Haidle and Storck, 1966; Inderlied and Sypherd, 1978; Larson and Sypherd, 1974; Lubberhuson et al., 2003; Ruiz-Heirera, 1985).

However, it has been shown that terminal budding yeast cells could be induced from *M. circinelloides* (Omoifo, 2005). Terminal budding yeast was not the sole morphology induced, but preliminary study showed that *M. circinelloides* could assume sigmoid growth habit, which is typical of *Saccharomyces cerevisae*. This indicated that at the cellular level, biochemical and molecular activities including signals transduction and mitotic divisions similar to what obtains in the model eukaryotic *S. cerevisiae* were possibly stimulated during the growth of *M. circinelloides* as yeasts.

As occurs in *S. cerevisiae* (Carman and Henry, 1989, 1999; Exton, 1994), plasma membranes of multipolar budding yeastlike cells of *M. racemosus* (Howe et al., 2002) and polar budding yeast cells of *Candida albicans* (Kerridge et al., 1976) have phospholipid constituents. Thus, they could also be said to be constituents of yeast cells, which were induced from sporangiospores of the mucoraceous form-genus, *Dimorphomyces pleomorphis* strain C13, with culture collection specimen number W5132B (IMI, 1996), in glucose-substrate multiionic broths (Omoifo, 1996). But when inositol was incorporated into the medium, growth magnitude decreased by 5.6% and morphology induced included terminal budding yeast cells, which predominated, multipolar budding yeastlike cells and conidia (Omoifo, 2005). On the other hand, biomass upped by 82.63% when sporangiospores of a related strain, *D. diastaticus* strain C12, specimen number W5132A, was the subject of study (Omoifo, 1997). Unlike *D. pleomorphis* strain

C13, IMI W5132B, induced morphologies during the cultivation of *D. diastaticus* strain C12 IMI W5132A, included septate mycelia, enterothallic- and holothallic conidia and a preponderance of nucleates, which were glistening and restive, assuming primordial yeast form (Omoifo, 2003, 2005). But optimal size yeast cells were not induced, as was the case with *D. pleomorphis* strain C13, IMI W5132B. It was noteworthy that *D. diastaticus* strain C12, IMI W5132A, proved to be maltose substrate-requiring uracil auxotroph for terminal budding yeast induction (Omoifo, 1997, 2003). The nucleates constituted a transient phase resulting from reorganization of protoplasm from which the growth sphere wall lysed and subsequently converted to protoplasts from which yeast morphology developed (Omoifo, 1996, 2003, 2005).

As part of an effort to elucidate the intrinsic factors that influence sporangiospore-yeast transformation, an hypothesis known as *sequential sporangiospore-yeast transformation* (SSYT) hypothesis (Omoifo, 2003) was formulated. Key elements of the hypothesis include occurrence of transmembrane-pH-gradient, synchronous DNA replication leading to cytosolic nucleation, *de-novo* structural build-up, intracellular signal transduction, and fermentative metabolism with consequent cell wall biogenesis and utilization of Zn^{2+}, as chaperon for molecular activities.

This SSYT hypothesis related ionic movement through membranes to structural modifications that gave rise to yeast cell induction (Omoifo, 2003). Such premise was derived from pre-logarithmic growth study of transforming sporangiospores (Omoifo, 1996, 1997). It was clearly shown that at lag phase, when growth spheres formed, there was Na^+ extrusion from intracellular medium, with simultaneous K^+ influx, a process reversed as cytosolic nucleates transformed to, and subsequently induced to, terminal budding yeast, in multiionic medium (Omoifo, 1996, 1997, 2003).

Since Zn^{2+} has been highlighted as a main player in the biochemical and molecular response of the organism with sequentially changing morphology in the synthetic growth milieu in the SSYT model (Omoifo, 2003), its role needs to be examined. Zn^{2+} was constituent of the medium that induced budding yeast from sporangiospores of filamentous microorganisms in several studies (Omoifo, 1996, 1997, 2003, 2005). It is known to play significant role at the cellular and molecular levels. For instance, it is part of the structural component of regulatory proteins, protein kinase C (PKC, Larsen and Sypherd, 1974), participates in gene regulation, such as DGPP phosphatase (Han et al., 2001), or would be found active in enzyme expression (Iwanyshyn et al., 2004).

This study reports the effect of varying levels of Zn^+ on the growth and morphology of *M. circinelloides*. Since myoinositol (myo) is involved in the generation of second messengers via formation of crucial phospholipids (Berridge, 1984; Carman and Zeimetz, 1996; Kikkawa et al., 1985), we also test its effects and show that it enhanced the induction of yeast cells from the microorganism.

MATERIALS AND METHODS

Chemicals

All chemicals were of reagent grade and obtained from the British Drug House, Poole, England except Bacto peptone (Sigma), myoinositol (Sigma) and yeast extract (Oxoid).

Fungal strain and maintenance

M. circinelloides Tieghe used in this study has been used in several studies (Omoifo, 2005). It was maintained on glucose-yeast extract-peptone agar (GYPA: 10-0.3-5.0 g/l) slants. Fresh culture was prepared on Petri dish unto which 20 ml of agar was poured and used after 7 days of growth.

Inoculum preparation for growth studies

A 7 day old culture of M. circinelloides was flushed with sterile deionized distilled water and a sterile glass rod gently passed over the fungal growth so as to dislodge the spores. The suspension, poured into centrifuge bottles, was washed with 3 changes of sterile deionized distilled water in an MSE 18 centrifuge at 5000 rpm for 7 min at 25°C. Spore count was taken with a Neubauer hemocytometer and was made up to 10^6/ml in sterile deionized distilled water.

Reagents and culture media

Procedure for preparing the base medium has been described (Omoifo, 1996) but since levels of myo were to be tested, these were prepared separately. Preliminary studies were carried out using various levels of myo and zinc. Thereafter, those levels, which had the greater impact on sporangiospore-yeast transformability of the organism, were chosen for this experiment. Each of duplicate flasks of the synthetic broth was incorporated with the various concentrations of myo (1.0, 2.0, 3.0 mM) and zinc (0.20, 0.25, 0.50 mM), in various combinations. The pH was adjusted to 4.5 with 2 N NaOH or 1 N HCl using a Cole-Parmer pH Tester model 59000. 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 (10^6/ml) was inoculated into each culture flask using a 0.5 ml suction pipette in a laminar flow chamber model CRC HSB-60-180. Before each inoculation the spores were kept in suspension by shaking for 30 s and the broths also shaken for 30 s after each inoculation before transferring to a preset, 20°C, cooled growth chamber. At 24 h intervals the culture flasks were brought to the chamber and 10 ml withdrawn from each culture flask, with sterile pipettes, one for flask, into factory-sterilized plastic sample tubes. The culture flasks were thereafter returned to the growth chamber. The samples were kept at –18°C until further analysis.

Table 1. Treatment combinations, biomass estimates with their respective standard errors, and form of growth during the cultivation of *M. circinelloides* in synthetic broth for 120h at pH4.5 and 20°C, ambient.

Treatment	Mean biomass	Form of growth
Zn: Myoinositol	at 520nm	
Control	0.434 ± 0.088	Y, Hc, $Mf_{s+ns,}$ P
0.20mM: 1.0mM	0.397 ± 0.085	Y, N, Gs
0.20mM: 2.0mM	0.411 ± 0.114	Y, Ec, Hc, Bl
0.20mM: 3.0mM	0.461 ± 0.153	Y, Hc, Mf_{s+ns}
0.25mM: 1.0mM	0.587 ± 0.206	Y, Hc, Mf_{s+ns}
0.25mM: 2.0mM	0.716 ± 0.195	Y, Hc, Ec, Mf_{s+ns}
0.25mM: 3.0mM	0.701 ± 0.189	Y, Hc, Mf_{s+ns}
0.50mM: 1.0mM	1.006 ± 0.534	Y, Hc, Ec, Mf_{s+ns}
0.50mM: 2.0mM	1.083 ± 0.304	Y, Ec, Hc, Gs, Mf_{s+ns}
0.50mM: 3.0mM	0.800 ± 0.220	Y, Mf, Hc, Mf_{s+ns}

Key Bl, blastospore; Ec, enterothallic conidia; Hc, holoblastic conidia; $Mf_{s+ns,}$ septate + nonseptate; Gs, growth sphere; N, granular particle or nucleates.

Table 2. A nested model analysis of variance of growth data of induced yeast cells of *M. circinelloides* obtained by measurement of O. D. at 520nm.

Source of variationnnon	DF	SS	MS	F-pr.
Treatment	9	5.4996	0.6111	<.001*
Time	4	13.1016	3.2754	<.001*
Treat. Time	36	6.3926	0.1776	0.187
Residual	49(1)	6.6348	0.1354	
Total	**98(1)**	**31.6274**		

*Significant at p < 0.001 probability level.

Biomass determination

The absorbance was obtained at 520 nm, using a Camspec M105 spectrophotometer (Cambridge, UK). The wavelength was chosen because of the greenish colour impact with the incorporation of $FeSO_4$.

Statistics

Experimental data was subjected to a nested model analysis of variance (ANOVA) test and considered significant if p<0.05 and comparison between means was performed using the Genstat 5 package.

RESULTS AND DISCUSSION

The combined effect of zinc and myo on biomass of the microorganism and its characteristics manifested under the different treatments were shown in Table 1. The least amount of growth occurred not in the control culture, but at the 0.20 mM Zn^{2+} : 1.0 mM myo treatment, while the higher biomass was at 0.5 mM Zn^{2+}- levels. In this study, the treatment at 0.25 mM Zn^{2+} : 1.0 mM myo had intermediate biomass. As the standard error showed, there was greater variability at the 0.5 mM Zn^{2+} treatments.

Analysis of variance at p<0.001 showed that individual treatments and time made important impact on the manifested characteristics, including magnitude of, and form of growth of, the microorganism; but the interactive element of treatment and time did not (Table 2). Means separation using the l. s. d. p<0.001, 0.3307 gave four homogenous subsets (Table 3). Treatment with myo at the 0.25 mM Zn^{2+}- level separated into two subsets, with the combination at 1.0 mM myo being distinct from the rest two sub-levels. Terminal budding yeast cells which were induced in all the treatments, was the most predominant cell type (Table 1). Other morphological forms included holoblastic and enterothallic conidia, septate and non-septate filaments, which did not form meshwork, and growth spheres. Protoplasts were observed in the control cultures but nucleates were also present at the 0.20 mM Zn^{2+} - 1.0 mM myo treatment. However morphological response at the highest level of Zn^{2+} treatment had copious presence of branched septate mycelia. Mycelia branches, which were not long, had apical ends frequently modified into bulbous structure with the cytoplasm appearing granular.

Figure 1 showed biomass profiles in the different zinc : myo treatments. *M. circinelloides* assumed sigmoid growth habit in control tests. Two optima occurred with 0.20 mM Zn^{2+} : 1.0mM myo treatment, the first well defined while the 2nd was still rising at termination of experiment. When this level of Zn^{2+} was combined with 2.0 mM myo, growth profile was more or less a straight line; at 3.0 mM myo, the lag was unusually long (72 h), but only one optimum exhibited. Apparently perfect sigmoid patterns occurred at 0.25 mM Zn^{2+} incorporation. Two optima occurred at the 0.5 mM Zn^{2+} levels with the

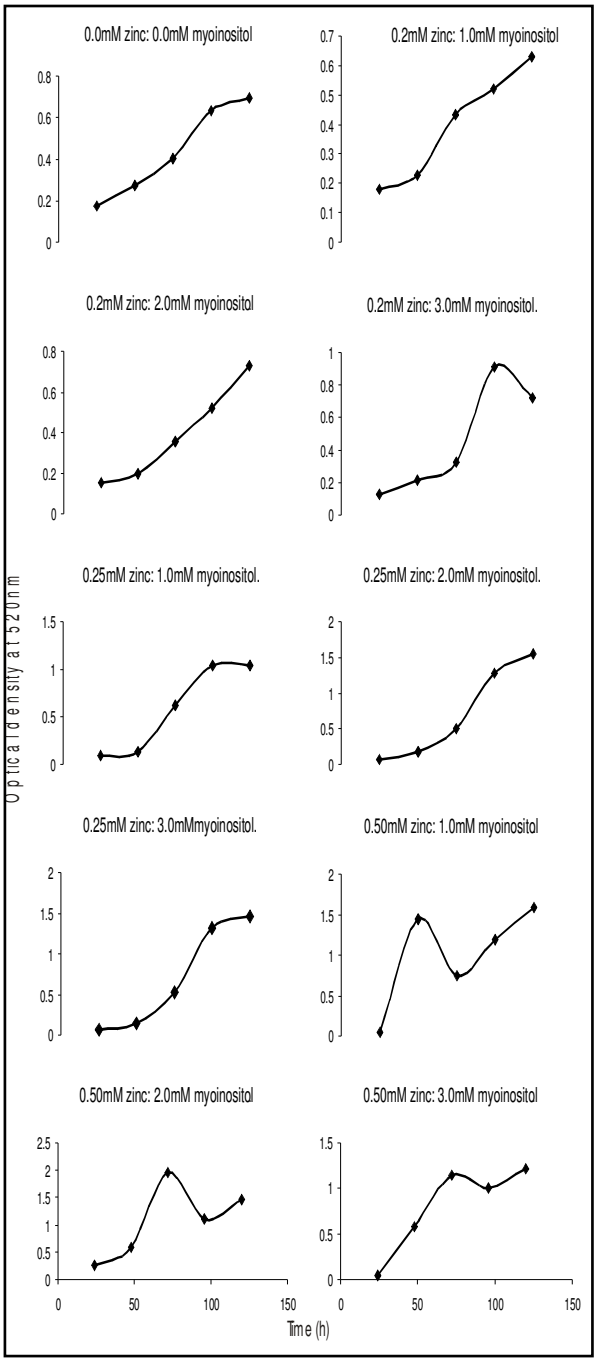

Figure1. Biomass profiles of *Mucor circinelloides* cultivated in synthetic broth incorporated with varying levels of zinc and myoinositol.

Note: Two growth patterns were prominent in these treatments. Firstly, a 2-phase growth, with the first peak being contributed by determinate thallic growth and growth sphere-released cytosolic nucleates and the second peak contributed principally by terminal budding yeast cells. Secondly, a sigmoid growth habit with the traditional partitioning into lag, exponential and stationary phases. Although thallic expressions were observed in all the treatments, they were extremely scanty in the 0.25mM zinc: 2.0mM myoinositol treatments.

Table 3. Homogenous subsets of mean biomass estimates obtained during sporangiospore-yeast transformation of *M. circinelloides* cultivated in synthetic broth for 120h, pH4.5 at 20°C, ambient.

Treatment, Zn: myoinositol	Mean
Subset 1	
0.5mM: 1.0mM	1.006
0.5mM: 2.0mM	1.083
0.5mM: 3.0mM	0.800
Subset 2	
0.25mM: 2.0mM	0.716
0.25mM: 3.0mM	0.701
Subset 3	
0.25mM: 1.0mM	0.587
Subset 4	
Control	0.434
0.20mM: 1.0mM	0.397
0.20mM: 2.0mM	0.411
0.20mM: 3.0mM	0.461

Means were separated using l.s.d., $p < 0.001$, 0.3307

1.0 mM myo level reaching its first optimum earlier (48 h) than other levels.

The occurrence of yeast cells in the control studies indicated that neither zinc nor myo was the primary inducers of the yeast morphology. That the growth profiles followed the well-known growth pattern in the control and 0.25 mM Zn^{2+} tests also indicated that physiology of the organism in such treatments is within the ambit of predictable partition of lag, exponential, stationary and death phases. Hence the relative growth rates could be obtained in treatments with such profiles (Figure 2). The first peaks of the double optima-treatments reflected morphological conversion where after thallic growth, cytoplasm turned granular, thereafter extruded after thallic cell burst (Omoifo, 2005). These then convert to protoplasts and subsequently yeast form (Omoifo, 1996; 2003; 2005), manifested in different sizes and shapes; the second upsurge in growth in these treatments has been attributed to this effect (Omoifo, 2005) and probably accounted for the higher biomass at 0.50 mM zinc treatments, which also showed greater variability.

It is noteworthy that zinc and myo supplementations enhanced biomass production, as there were valid increases over the control mean data (Figure 3). Although the effect of myo seemed additive to that of zinc, results of mean biomass separation showed that growth was better enhanced at 2.0-3.0 mM myo supplementations. At the 0.25 mM zinc level, there was no additional benefit derived in using the higher level of myo, 3.0 mM. Therefore, Zn^{2+} at 0.25 mM to myo at 2.0 mM met the requirements for enhanced level of yeast cell

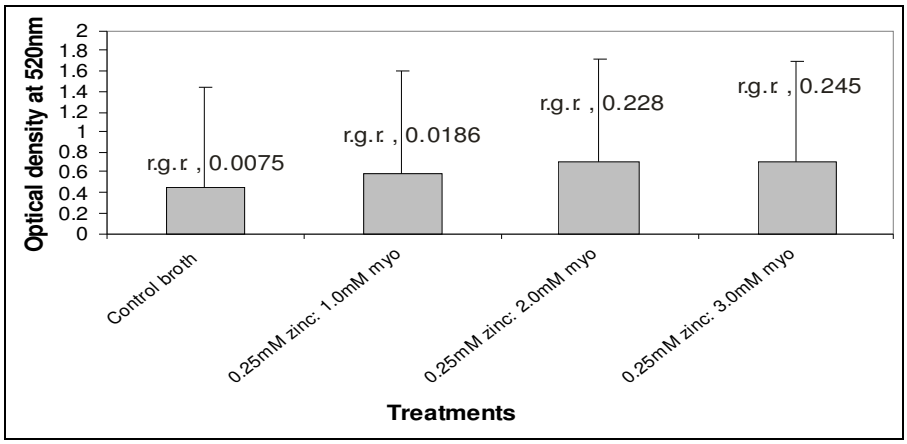

Figure 2. Mean biomass and relative growth rates of *M. circinelloides* cultivated in treatments in which sigmoid growth habit was expressed.

Note Cells in the broth were predominantly terminal budding yeasts, which were transformed from aerobically produced sporangiospores by the filamentous microorganism. Other morphologies observed in the broths, although minimal in contrast to the observation in the 2- phase growth pattern described in fig. 1, were enterothallic- holothallic- and holoblastic conidia. Conidial subtypes were scantier in the 0.25mM Zn^{2+}: 2.0mM myoinositol treatment. Hence the decision that yeast cell in the treatments exhibited the sigmoid growth habit, in the fashion of *S. cerevisiae*. Perhaps the thallic subtypes influenced the higher relative growth rate (r.g.r.) at exponential phase in the 0.25mM Zn^{2+}: 3.0mM myoinositol treatment.

inductions of *M. circinelloides* in synthetic broth. Although terminal budding yeast cell was the predominant morphology at this level of treatment, other morphologies observed include holoblastic conidia and mycelia fragments.

The study indicates three- pronged relationships between the organism and the material environment. One result of this correlation is that following sporangiospore inoculation structural modifications thus generated transient morphologies but yeast form was the eventual phenotypic expression. When thallic growth occurred it did not form a meshwork. The height of formation of this constituted the first optima of the biomass profile. But this occurred in the less cooperative treatments for yeast inductions in contrast to the more cooperative ones where sigmoid growth pattern was described (Omoifo, 2005). Another important result is that small changes in the monovalent ions (H^+, K^+, Na^+) caused alterations in the phenotypic expression. As the study of Omoifo (1996) showed, minute but incremental drop in bulk medium pH considered vectorally unidirectional, in which case the intracellular pH increased, thus providing environment for synchronous DNA replication (Rozengurt, 1986; Ryan and Ryan, 1972), altered the spore form to the growth sphere; a subsequent lyses of cell wall occurred, that is, a structural effect, arising from proton-gradient equilibrated lysozyme activities (Maurel and Douzou, 1978) and nucleates released, which was optimal at pH 4.5 (Omoifo, 2005); bulk medium pH thereafter built up, meaning a unidirectional H^+ influx into the intracellular medium and this coincided with protoplast formation (Omoifo, 1996). It was inferred that the apparent proton movement played key role in both structural and molecular modifications of the evolving cell.

A study conducted using a range of concentrations of K^+ showed that proton release intensity from intracellular medium of *M. circinelloides* was highest at 7.35 mM K^+, where there was optimum biomass production and induced yeast cells dominated the medium of growth (Omoifo, 2005). On the reverse therefore, there was increase in the pH of the intracellular medium and enhanced K^+ uptake (Borst-Powells and Peters, 1977; Camacho et al., 1971; Ryan and Ryan, 1972) by protoplasts within which intracellular differentiation occurred (Chalazonitis and Fishbach, 1980). Studies have shown that K^+, which is exchanged for Na^+ (Glynn and Karlish, 1975; 1975b;; Lingrel and Kuntzweiler, 1994; Tonomura, 1986), is prerequisite for the formation of protoplasts (Omoifo, 2005). The influx of K^+ therefore meant increased depletion of Na^+ during protoplast formation. Previous results (Omoifo, 2005) have shown that the Na^+-K^+ antiport movement reversed as consequence of protoplast formation, and as yeast morphology induced. Furthermore, it was not just the presence of Na^+ ion in the intracellular medium of protoplast, but specifically its influx rate, or accumulation that triggered yeast form development (Omoifo, 2005) Considering H^+-substrate symport at protoplast formation (Omoifo, 1996, 1997, 2003, 2005), thereby making available in the cytosol fermentable carbon substrate that called for specific biochemical and physiological direction, this could be fermentative metabolism in the buffered

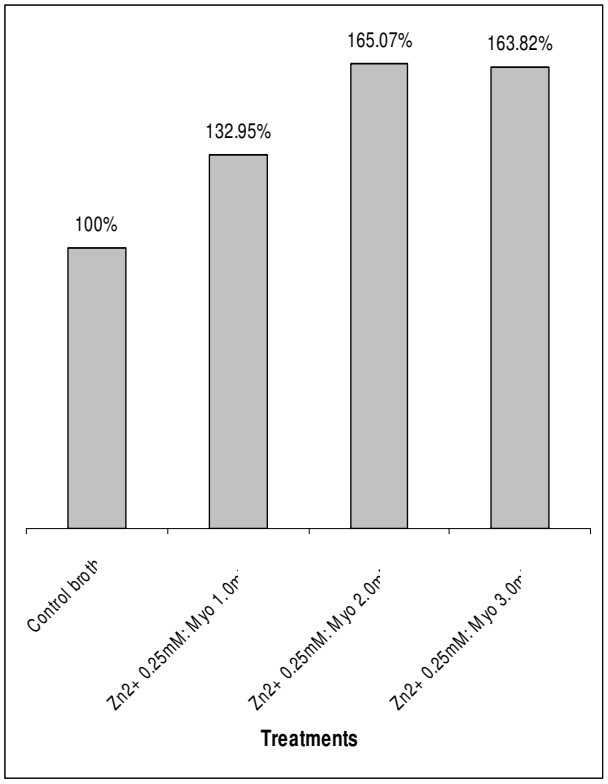

Figure 3. Percent increase from control mean biomass of *M. circinelloides* as predominantly yeast cells in synthetic broth where sigmoid growth habit was expressed.

Note One million sporangiospores were inoculated into each medium and, in a classical demonstration of dimorphism, these transformed to terminal budding yeast cells and determinate thallic subtypes but neither sporophore nor sporangium was produced. Microscopic examination showed that the morphology of note was the yeast form, which was responsible for the large increases in total biomass.

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broth. Glucose is also inducers of the enzyme, glucokinase (Merrill and Pitot, 1986). In a favorable microenvironment, the primer Glucose-1-6-bisphosphate possibly formed, would then generate glucose-1-phosphate, hence the induction of myoinositol-1-phosphate synthase, the rate limiting step in inositol biosynthesis (Agam et al., 2002; Ju et al., 2004); this could spell redox reactions thus leading to the synthesis of inositol phosphate, important for the execution of the phosphatidyl inositol (PI) cycle (Abeles et al., 1992; Alberts et al., 1987). Assuming the occurrence of these reactions in our system, then phospholipids, constituents of the plasma membranes, were generated through the PA-CDP or Kenedy pathways (Iwanyshyn, et al., 2004).

Iwanyshyn et al. (2004) showed that in *S. cerevisiae* major phospholipids including phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine were still synthesized in the absence of zinc and myo, although there was reduction in their contents. However, phosphatidylinositol content was upped by as much as 29%. Thus we deduce that in our study without zinc or myo incorporation, phospholipids were generated and there was also elevated phosphatidylinositol content. These then would participate in the PI cycle to generate second messengers (IP_3 and DAG), which effect signal transduction at the sub cellular level.

Since individual messenger elicits specific response (Abeles et al., 1992; Bell and Burns, 1991; Kikkawa et al., 1985; Lalli and Sassone-Corsi, 1994, Rozengurt et al., 1984), IP_3 stimulates the translocation of PKC to its indispensable membrane-bound cofactor, phosphatidylserine, after which it binds stereo specifically to *sn*-1, 2 DAG, which occasions the release of the catalytic subunit of PKC. This relocates to the membrane bound adenyl cyclase and thus triggers the release of cAMP for its kinase cascading and mitogenic roles (Larsen and Sypherd, 1974; Rozengurt et al., 1984). In the case of PKC migration, Korichneva et al. (2002) demonstrated, using confocal microscopy that PKC mobilization occurred as a result of efflux of bound zinc ions from its regulatory tetrahedral complex. In our control study without zinc supplementation migration could have been due to other divalent ions. For as Paveto et al. (1975) showed, Mn^{2+} could replace Zn^{2+} up to 2% during the conversion of *Candida albicans* from the filamentous to the yeast form. On the other hand, Bartnicki-Garcia and Nickerson (Bartnicki-Garcia and Nickerson, 1962b) showed that Fe^{2+} and Cu^{2+} were effective in reverting filamentous growth habit to yeastlike form of *M. rouxii*. $Fe^{2+,}$ Mn^{2+} and Cu^{2+} were incorporated into the synthetic broth used in the present study.

Zn^{2+} influx into the cytoplasm is electrogenic (Zhuang et al., 1995) being transported through the divalent cation transport proteins (Colvin et al., 2000), or other zinc transporters (Alberts et al., 1987; Xing et al., 1996). The ions, spatially located in the zinc-finger motif of the PKC, tetrahedrally coordinate 6 cysteine and 2 histidine residues in the regulatory domain, and respond stoichiometrically to DAG binding (Lalli and Sassone-Corsi, 1994). Korichneva and colleagues (2002) showed that when DAG converges on the $Zn2^{+}$-finger regulatory domain, the structure collapses and the ions released into the cytosol, making the PKC change conformation and hence become active catalytically; the more ions released from the structure and into the cytosol, the higher the catalytic ability.

In the present study, biomass responded differentially to treatments with different levels of Zn^{2+}. This confirmed unpublished observations where Zn^{2+} was the sole factor varied. Of note in that study, sigmoid pattern was not exhibited in the 0.25 mM Zn^{2+}- mediated broth in which biomass and the proportion of yeast formed were optimal. In the present study, and of the treatments with sigmoid growth habit, growth mean was optimal at 0.25 mM Zn^{2+}: 2.0 mM myo, which had more than 65%, rises over the

control biomass. This indicated an elevated rate of influx of Zn^{2+} into the cytoplasm. Perhaps too, when appropriate level of zinc had accumulated in the cytosol, the requisite yeast form PKC isoform, which is distinct from the filamentous type (Aquino-Pinero and Rodrigue-del-Valle, 1997), was mobilized to its cofactor binding head group, phosphatidylserine, and with the induction of DAG and IP_3 promoted by the incorporated myo, could progressively fulfill their mitogenic and cell proliferation functions. Independent observations also showed that the exogenous application of 1.0-3.0 mM myo promoted sporangiospore-yeast transformability of *M. circinelloides*. That there was lower biomass recorded at lag phase in the 3.0 mM myo-mediated broth indicated that the processes of conversion of spore to growth sphere, which then lysed, followed by protoplast formation and subsequent yeast morphology induction was slower when compared to the 2.0 mM myo levels. Although induced yeast cell turnover at exponential phase in the presence of 3.0 mM myo was greater than that at 2.0 mM myo by 0.0017 units, the turnover rate (μ) at the stationary phase when cells normally die decreased further than that of the latter (compare 3.0 mM myo, μ = 0.0058; 2.0 mM myo, μ = 0.011). This indicated that the autophagic entry of cytosolic contents into vacuoles for degradation (Iwanyshyn et al., 2004) was heightened at the 3.0 mM myo level. The slower conversion process at lag phase, considered along with the faster rate of entry into the death phase, and this coupled with the fact that mean biomass in the 3.0 mM myo treatment had the same significance with, showed that this level of treatment provided no added value to the 2.0 mM myo treatment. However, this study emphasized that $ZnSO_4$ and myo were precursors to intracellular factors as second messengers, used for signal transduction, which elevated the buffered environment-specific terminal budding yeast induction capability and determinable growth pattern of the synthetic broth used in the incubation of sporangiospores of *M. circinelloides*.

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