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Induction of yeast cells from sporangiospores of *Rhizopus stolonifer* was inhibited by sodium fluoride

C. O. Omoifo^{1*}, R. E Ohenhen² and R. A Elemonfo²

¹Department of Crop Science, Ambrose Alli University, Ekpoma, Nigeria.

²Department of Microbiology, Ambrose Alli University, Ekpoma, Nigeria.

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This study was aimed at determining the effect of the glycolytic inhibitor, sodium fluoride (NaF), on induced yeast cells. *Rhizopus stolonifer* was cultivated in synthetic broth for 120 h, at pH 4.5 and ambient temperature of $28 \pm 1^\circ\text{C}$, using sporangiospores as inoculum. Growth was in discrete units, which were sedimented. Microscopic examination revealed induction of neoplasts, protoplasts and yeast cells, but biomass profiles (OD, 625nm) did not exhibit sigmoid pattern in control or glycolytic inhibitor-NaF incorporated broths. A two-way analysis of variance showed that NaF and its interaction with time significantly affected growth ($p < 0.05$). Means separation was into three subsets, with 20 mM (subset 1) having the highest biomass and least occurred at 5 mM, which was comparable to 30 mM, control and 10 mM (subset 3). A further study revealed effect of application time of NaF at two levels (20 and 10 mM) on morphological expression. Three main effects were observed: (a) complete inhibition of yeast induction, (b) delayed induction of yeast cells and (c) apoptosis of induced yeast cells. In the complete absence of yeast cells, protoplasts were copiously produced. However, when yeast cells were induced prior to NaF challenge, the inhibitor caused death of cells. Hence, it was suggested that induction of terminal budding yeast cells was closely associated with glycolysis. Further studies are recommended in order to examine the contribution of individual inductive enzymes including those of glycolysis, during the transformation process.

Key words: *Rhizopus stolonifer*, sporangiospores, protoplasts, yeast induction, sodium fluoride, apoptosis.

INTRODUCTION

Fungal dimorphism is the ability of a fungus to exist in two different morphological forms whereby it exhibits filamentous or yeast-like growth habit. This occurs as a result of changes in environmental conditions (Romano, 1966). The Mucorales contains a number of species that are known to undergo this dual morphogenetic change. They include the much-researched *Mucor rouxii* (Bartnicki-Garcia, 1968; Bartnicki-Garcia and Nickerson, 1962a, b, c, d; Schulz et al., 1974), *Mucor circinelloides* (Lubberhusen et al., 2003; McIntyre et al., 2002), *Mucor genevensis* (Rogers et al., 1974), *Mucor racemosus* (Mooney and Sypherd, 1976), *Mucor pusillus* (Fisher, 1977), *Mucor hiemalis* (Mysyakina and Funtikova, 2000),

Cokeromyces poitrasii (Price et al., 1973), *Mycotypha* spp. (Hall and Kolankaya, 1974). In each of these cases, the alternative to filamentous growth is yeast-like form, which has a globose mother cell that gives rise to numerous buds from different loci by blastic action. Although such units are discrete, the form of growth is different from that of *Saccharomyces cerevisiae*, which reproduces asexually by terminal budding.

However, *M. circinelloides* has been shown to exhibit terminal budding yeast growth in well-defined synthetic medium (Omoifo, 2006a, b; Omoifo and Awalemhen, 2012). In glucose substrated minimal medium, the organism exhibited polymorphic existence occurring as

*Corresponding author. E-mail: coomoifo@yahoo.com.

holothallic-, holoblastic-, thallo-arthric conidia and terminal budding yeast cells, which was preponderant. Myoinositol (myo) enhanced the proportion of yeast cells induced in the multiionic medium, which was optimum at 2 mM myo but growth did not follow a sigmoid pattern (Omoifo et al., 2006). Sigmoid growth was however obtained when zinc was incorporated into the medium of cultivation (Omoifo and Omamor, 2005). Studies had previously shown that the incorporation of zinc in the growth medium led to the conversion of *M. rouxii* to yeast-like cells (Bartnicki-Garcia and Nickerson, 1962b) and *Candida albicans* to yeast cells (Yamaguchi, 1975).

Rhizopus stolonifer, which is also a Zygomycete like *M. circinelloides*, was said to be incapable of morphogenetic inter-conversion (Bartnicki-Garcia and Nickerson, 1962b). We have shown that *R. stolonifer* also exhibits dimorphism in xylose substrated minimal medium where several morphological forms were induced. These included septate mycelia, moniform hyphae that apically produced yeast mother cells that ceded, thereafter becoming terminal budding as well as spore-derived terminal budding yeast cells; these occurred beside coenocytic mycelia simultaneously expressed on exposure of the broth-bound septate mycelia to the headspace/atmosphere (Omoifo, 2011a). Thus, like *M. circinelloides*, *R. stolonifer* exhibits saccharomycetous growth habit at a stage in its life cycle. Perhaps *R. stolonifer* will exhibit sole yeast morphology in the specific medium used for the induction and growth of terminal budding yeast cells of *M. circinelloides* by Omoifo (2006a, b). This proved to be the case where induced yeast cells of *R. stolonifer* was found to assume sigmoidal growth habit at pH 5.0 (Omoifo, 2011b), as distinct from *M. circinelloides* that exhibited similar morphology and optimum growth at pH 4.5.

However, several transient morphologies have been observed in the conversion of spores to yeasts. These include growth/germ spheres which thereafter lyse thus releasing granular/neoplastic units and protoplasts. The occurrence of the transient forms was said to be pre-logarithmic (Omoifo, 2009). It was also proposed that spore-to-yeast conversion occurred at acidic level where an electrogenic biphasic pattern is established, controlled by H⁺ release ability of the intracellular medium of the transforming microorganism (Omoifo, 2012; Omoifo and Awalehmen, 2012). Thus, a sequential sporangiospore-yeast transformation (SSYT) hypothesis was proposed as a format for studying the conversion process (Omoifo, 2003). This led to the model development where the transient form, protoplast, assumed central significance (Omoifo, 2009).

Studies in our laboratory have shown that potassium (K⁺) was necessary for the formation of protoplasts since a thorough examination of control cultures did not reveal these units. But this was possible only in proton-directed intermedial communicating multiionic system. Protoplasts when formed could be cylindrical, ovoid, short rod and in

single or double and, or bacterial-like. From these yeast cells evolve, which could be globose, subglobose, pyriform, obpyriform or cylindrical (Omoifo, 2009). The yeast cells constituted the dominant morphology in the 1.0 K⁺ to 0.10 Na⁺ g/L modulated broth during the conversion of *M. circinelloides* (Omoifo and Awalehmen, 2012). In the aforementioned study, it was shown that protoplasts originated from two structures: growth/germ spheres and ruptured hyphal cells. When protoplasts, which were copiously obtained at 15°C, were transferred to solid cultures and incubated on the side bench, 28 ± 1°C, filamentous growth was induced (Omoifo, 2003). A role for aerobiosis was therefore implicated. Such physiological import had been suggested by other researchers, including Hall and Kolankaya (1974) and Schulz et al. (1974), but in each of the studies, multipolar budding yeastlike cells was obtained from spore-inoculums. It was therefore necessary to explore the physiological direction that led to terminal budding yeast development.

The objective of this study, beside further confirmation of the induction of terminal budding yeast cells from spores of *R. stolonifer*, was to examine the effect of the glycolytic inhibitor, sodium fluoride (NaF), on induced yeast cells. It showed that several transient morphologies occurred during the transformation process and that adoption of the yeast morphology was inseparable from the physiological process of glycolysis.

METHODOLOGY

Fungal strain and maintenance

The organism, *R. stolonifer*, used in this study was a gift from Mr. Frank Wizuzu, Principal Technologist, Plant Pathology Division, Nigerian Institute for Oil Palm Research, Benin City, Nigeria. It was maintained as glucose-yeast extract peptone (GYP: 10:03:5 g/L) solid cultures where it exhibited coenocytic 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 cultures and a sterile glass rod gently passed over the surface so as to dislodge the spores. The suspension was poured into sterile centrifuge tubes and spores were washed by centrifuging at 5000 rpm for 7 min at 25°C in an MSE 18 centrifuge. The supernatant was decanted, sediment re-suspended and further washed with two changes of deionized sterile 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 deionized sterile distilled water with the aid of tally counter.

Reagent and culture media

The minimal medium used in this study has been previously described (Omoifo, 2006b). Briefly, media were prepared per litre: glucose (Fluka Chemicals), 10 g; (NH₄)₂SO₄ (BDH), 5 g; K₂HPO₄ (BDH), 5 g; KH₂PO₄ (BDH), 5 g; MgSO₄ (BDH), 2 g; FeSO₄ (BDH),

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

Treatment (mM)	Mean biomass, standard error	Form of growth
NaF	O. D., 625 nm	
Control	0.143, 0.0014	Y
5	0.121, 0.012	N, P, Y
10	0.132, 0.014	P, Y
20	0.174, 0.007	P, Y
30	0.144, 0.013	P, Y
50	0.159, 0.018	P, Y

N, Nucleates; P, protoplasts; Y, terminal budding yeast cells.

0.1 g; NaCl (M&B), 0.1 g; CuSO₄ (BDH), 0.06 g; MnSO₄ (BDH), 0.065 g; ZnSO₄ (BDH), 0.06 g; uracil (Sigma), 100 mg; myoinositol (Sigma), 0.250 g. Media were prepared in 2000-ml beaker. Weights of buffer components 0.2 M Na₂HPO₄: 0.1 M citrate were obtained using H54Ar meter balance and added to the beaker. The pH was adjusted to 4.5 with 2 Normal NaOH or 1 Normal HCl using a Woonsocket model Ri 02895 pH meter in the 2000-ml beaker before dispensing 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 and 15 lb per square inch for 15 min.

Inoculation, growth conditions and sample collection

In brief, 1 ml of spore suspension was drawn and inoculated into each broth flask using a 0.5-ml rubber suctioned pipette. The inoculum flask was shaken at each operation so as to keep the spores well distributed in suspension. Each culture flask was then shaken for 30 s and thereafter incubated at 28 ± 1°C. At 24 h intervals the culture flasks were transferred to the sterile inoculating chamber, each shaken to keep the sediment in suspension and 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. The samples were kept at -18°C until analysis. Slides preparation was then made from each of the sample tubes for subsequent microscopic examination.

Two experiments were conducted: Experiment 1: To demonstrate the effect of NaF on biomass and the form of growth of *R. stolonifer* in synthetic broth. The inhibitor, NaF, at concentrations of 0, 5, 10, 20, 30 and 50 mM was incorporated into duplicate flasks containing 100 ml synthetic broth and flasks were labeled Run 1 and Run 2. The inhibitor was prepared into 100 ml flasks. Experiment 2: To show the effect of application time of NaF on the form of growth of *R. stolonifer* in synthetic broth. Two levels of NaF, 10 and 20 mM were used in this study. Slides were prepared for viewing by putting one or two drops of lactophenol in cotton blue on a glass slide and with the aid of a flamed wire loop; one drop from the culture flask properly shaken was then added and covered with a glass cover slip and viewed under a binocular microscope at × 400 magnification. The morphologies at different hours were recorded.

Biomass determination

Culture broth samples were thawed up to room temperature before biomass determination. This was done by measurement of optical density at 625 nm (Sharma et al., 1984). Absorbance was

determined with a Camspec M105 spectrophotometer CE 303 (Cambridge, UK). Triplicate determinations were made.

Statistics

Results were subjected to a two-way analysis of variance (ANOVA) test for the single factor and combined interactions, using the Genstat Discovery Edition statistical package. The combined factors are considered significant if *p*0.05; comparison between means was performed at *p*0.05.

RESULTS

Effect of NaF on growth

The effect of NaF on biomass of the microorganism and characteristics manifested under the different treatments are shown in Table 1. The least amount of growth occurred at 5 mM NaF, while the highest mean biomass was obtained at 20 mM NaF. As shown in the table, the control broth (s.e., 0.00014) had the least variability and the form of growth was solely terminal budding yeast cells. This level of variability was followed by the 20-mM NaF incorporated broth (s.e., 0.007); protoplasts were the main growth forms manifested. The boxplot of optical density measurement (Figure 1) shows the concise population of the microorganism; the data components were devoid of outliers and such population was symmetrical around zero in each of the NaF-treated broths. This implies that in this study, the parameters obtained followed the normal population distribution.

Furthermore, the median value in the 20-mM NaF treatment was higher than the others but lowest with the 5mM NaF treatment, which was apparently comparable with the control treatment. To test the significance of this, an analysis of variance of the growth data was obtained. A 2-way analysis of variance showed that NaF at *p*0.05, and NaF and its interaction with time at *p*0.001, had significant impact on the growth of the microorganism. Means separation, using l.s.d. at *p*0.05, 0.0325 gave

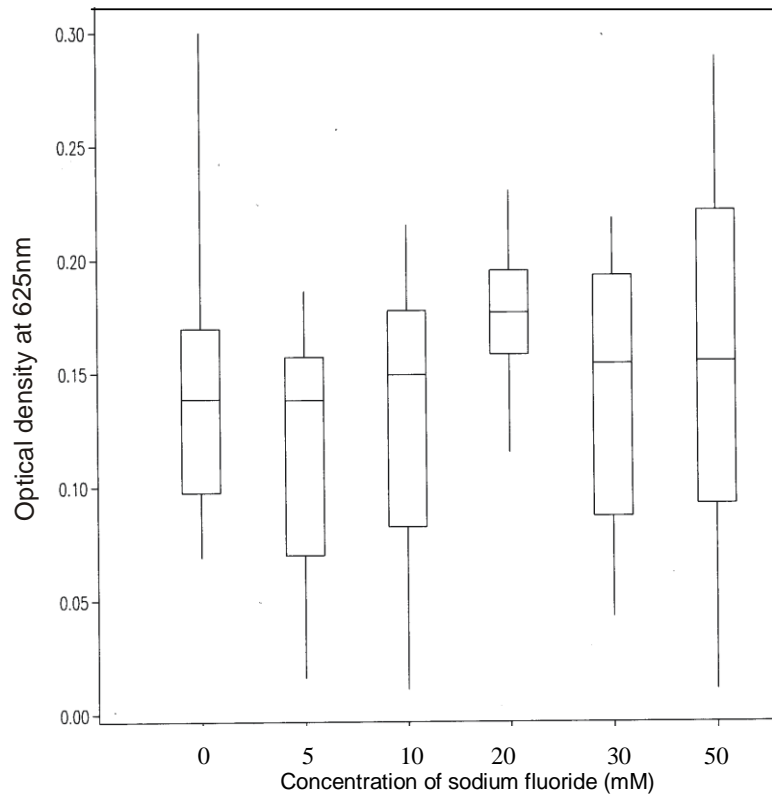


Figure 1. Boxplot of growth data of *R. stolonifer* cultivated in synthetic broth incorporated with different levels of NaF. The absence of outliers indicated that the growth of the organism in the broth followed the normal population distribution in spite of the distinct morphologies exhibited. These include neoplasts, protoplasts and yeast cells.

Table 2. A 2-way analysis of variance of growth data of induced yeast cells of *Rhizopus stolonifer* obtained by measurement of O. D. at 625 nm.

Source of variation	DF	SS	MS	F.pr
NaF	5	0.034753	0.006951	0.030*
Time	4	0.020613	0.005153	0.112
NaF × time	20	0.156971	0.007849	<0.001*
Residual	90	0.240502	0.002672	
Total	119	0.452840		

*Significant at $p < 0.05$.

three homogenous subsets (Table 2). Treatment with 20 mM NaF, which had the highest mean biomass, was distinct and this was followed by treatment at 50 mM (subset 2), while all the other treatments had comparable means (subset 3).

Figure 2 shows the biomass profiles of *R. stolonifer* cultivated in synthetic broth incorporated with varying levels of NaF. Although the growth profile in each of the treatments did not show a sigmoid growth pattern, yeast cells were induced. In the control broth, yeast cells were observed right from day 1 till termination of experiment.

The terminal budding yeast cells varied in shape; they were ellipsoidal, ovoidal and obpyriform. They also occurred at 20 mM NaF, but the terminal budding yeast cells diminished in number; in addition, protoplasts were observed. Moreover, they appeared purplish in contrast to the blue stain taken up by the yeast cells. As the concentration of NaF increased from the lowest level, protoplasts became more preponderant. In the media with 20 mM NaF treatment, mainly nucleates/neoplasts and protoplasts were observed up till 72 h but by 120 h, yeast cells of the various aforementioned shapes were

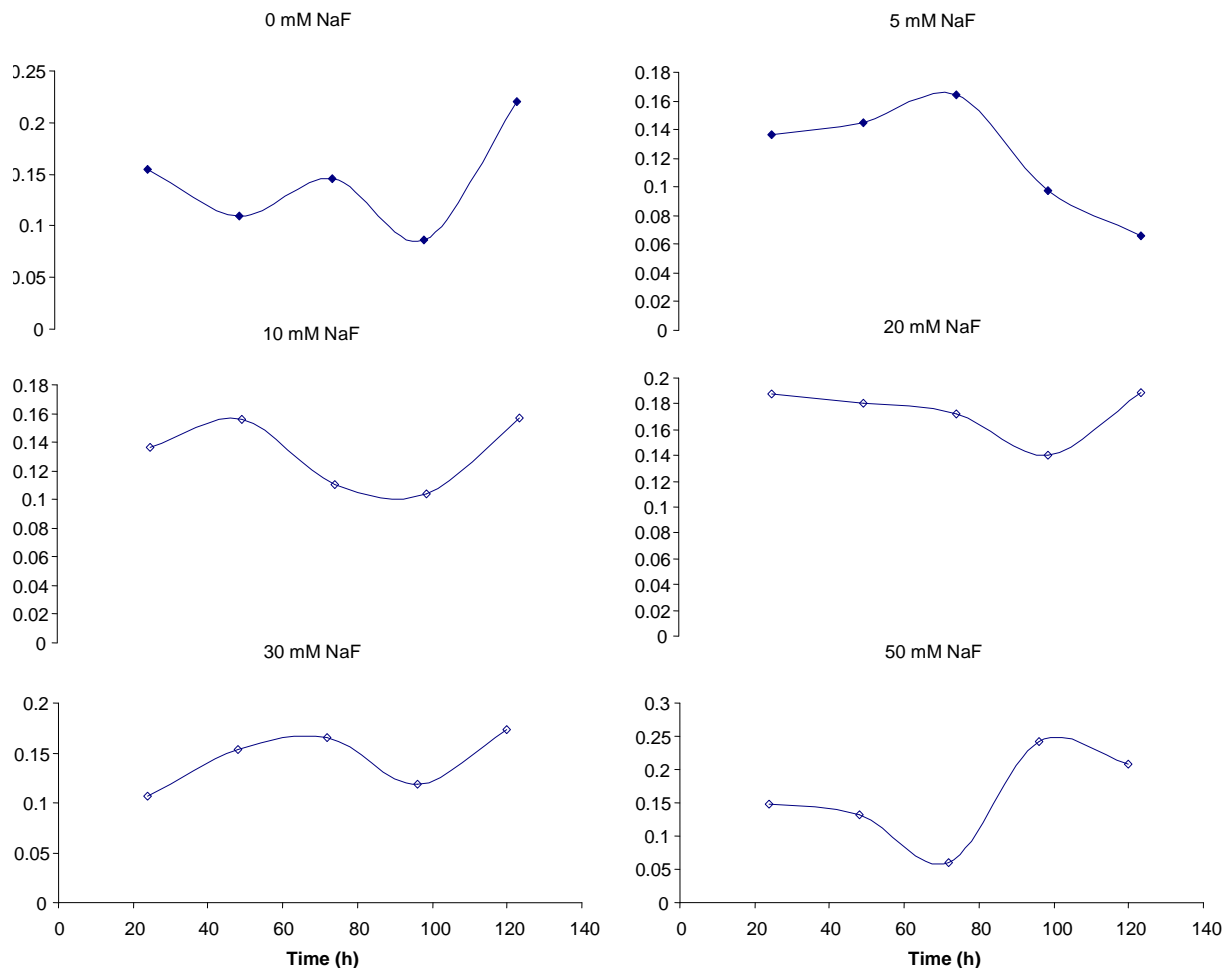


Figure 2. Biomass profile of *Rhizopus stolonifer* cultivated in synthetic broth incorporated with varying levels of sodium fluoride. The sigmoid growth habit was not exhibited in submerged cultures.

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

Treatment	Mean
Subset 1	
20 mM	0.174
Subset 2	
50 mM	0.159
Subset 3	
30 mM	0.144
Control	0.143
10 mM	0.132
5 mM	0.121

Means were separated using L.S.D., $p < 0.05$, 0.0325

seen; however induced yeast cells diminished with increase in NaF incorporation. Thus, yeast cells had very scanty presence at the 50 mM level.

Effect of application time of NaF on growth

It became necessary to find out what constituted the growth characteristics of the two extreme groups, that is, subsets 1 and 3 as reflected in Table 3. The boxplots of optical density at the two NaF levels is shown in Figure 3. It also showed that there was normal distribution of population during the period of growth. In order to find out at what stage in the conversion process of the microorganism that NaF was most effective, the inhibitor was incorporated into the broth flasks at different stages of growth of the microorganism. Analysis of variance of growth data showed that the individual treatments as well as interaction of the inhibitor with application time, and application time \times sampling time had significant impact on growth of the organism at $p < 0.001$ (Table 4). Separation of

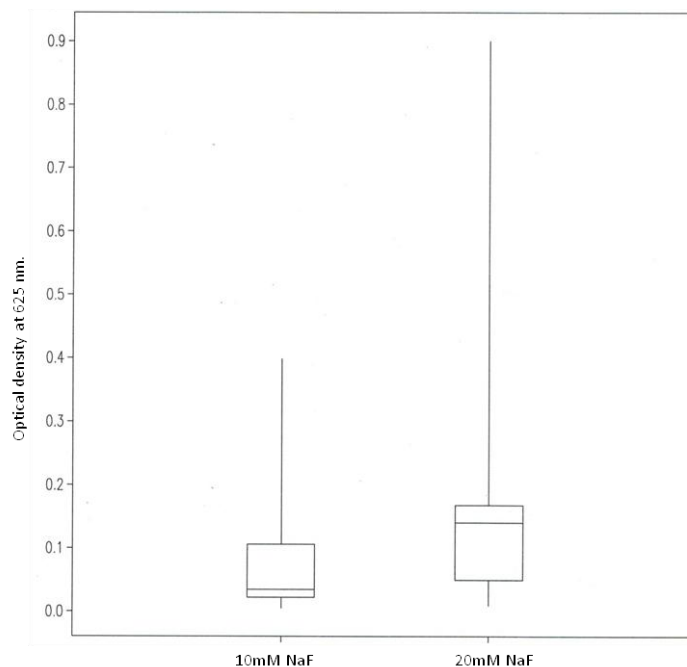


Figure 3. Boxplot of growth data of *Rhizopus stolonifer* cultivated in synthetic broth incorporated with 10 and 20 mM NaF. The 10 mM NaF effect was more profound in repressing the induction of yeast cells.

Table 4. A 2-way analysis of variance of growth data of *Rhizopus stolonifer* cultivated in synthetic broth incorporated with 10 and 20 mM NaF obtained by measurement of O. D. at 625 nm

Source of variation	df	ms	F.pr
NaF level	1	0.130014	<0.001*
Application time	5	0.039226	<0.001*
Sampling time	4	0.071328	<0.001*
NaF level × Application time	5	0.037448	<0.001*
NaF level × Sampling T	4	0.001558	0.825
Application time × Sampling time	20	0.016137	<0.001*
NaF level × Application Time × Sampling time	20	0.008345	0.008
Residual	180	0.004131	
Total	239	2.038199	

*Significant at $p < 0.001$.

the means obtained was into three subsets; simultaneous application gave the highest biomass. This was followed by application after 6 and 72 h as shown in subset 2, while the least biomass was obtained at application after 24, 36 and 48 h (Table 5).

Table 6 shows the growth rates of the organism when NaF was incorporated at different application times in the broths. The maximum growth rate was achieved when 10 mM NaF was applied 72 h after inoculation, but the least was at 24 h after inoculation. On the other hand, at 20 mM NaF maximum rate of growth occurred on application

72 h after inoculation and this was higher than the rate at 10 mM NaF comparable application time. However, the least growth occurred 36 h after inoculation, and this was negative in value; a similar rating with 6 h after inoculation.

Effect of application time of NaF on the occurrence of yeast cells

Although simultaneous application with inoculum had the

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

Treatment	Mean
Subset 1	
Simultaneous application	0.1559
Subset 2	
After 6 h	0.1042
After 72 h	0.1019
Subset 3	
After 48 h	0.0923
After 36 h	0.0713
After 24 h	0.0704

Means were separated using I.s.d., $p < 0.05$, 0.02836. Although broths inoculated with sporangiospores simultaneously with NaF application, i.e., zero application time, had the highest biomass, microscopic examination did not reveal any yeast cells, except nucleates and protoplasts, which predominated, throughout the period of observation.

Table 6. Growth rates of *Rhizopus stolonifer* as discrete units during 120 h of growth in synthetic broth.

Treatment	Sodium fluoride concentration	
	10 mM	20 mM
Growth rate (μ)		
Simultaneous application	0.0049	0.0102
After 6 h	0.0053	-0.0005
After 24 h	0.0020	0.0109
After 36 h	0.0042	-0.0009
After 48 h	0.0091	0.0094
After 72 h	0.0107	0.0125

$\mu = \log_{10}/h$ [Gibson et al., 1988].

highest biomass in the 10 mM NaF broths, microscopic examination did not reveal yeast cells throughout the period of observation. Morphological entities were cytosolic-derived neoplasts and protoplasts. At 6 h application time, morphological entities were mainly neoplastic units and well-rounded protoplasts, but the former was dominant. At 24 h application time, protoplasts were well-formed 72 h after inoculation and were the sole morphology recorded by 120 h of cultivation. Four morphological forms were observed 72 h after inoculation when application time was 36 h; these included neoplasts, protoplasts, thin monolithic filaments with apparent septation and yeast cells. The yeast cells were globose and scanty, but protoplasts dominated the broths even after 120 h of growth. Furthermore, at 48 h

application time, hyaline protoplasts and globose/ovoid terminal budding yeast cells although scanty, were observed 48h after inoculation. By 120 h, the yeast cell shape became more varied as obpyriform shape was also observed. However, neoplasts and protoplasts dominated the medium of growth.

When the application time was 72h, terminal budding yeast cells in single or short chains, and spheroidal, globose, or rod shaped were preponderant 72 h after inoculation. By 120 h of cultivation, some yeast cells appeared collapsed, and some of such remained as frass in the medium. There was apparent reduction in cell numbers. It was noteworthy that growth at 6 h application time had similar biomass with 72 h application time (Table 4, subset 2). Yet, there was no yeast cell observed

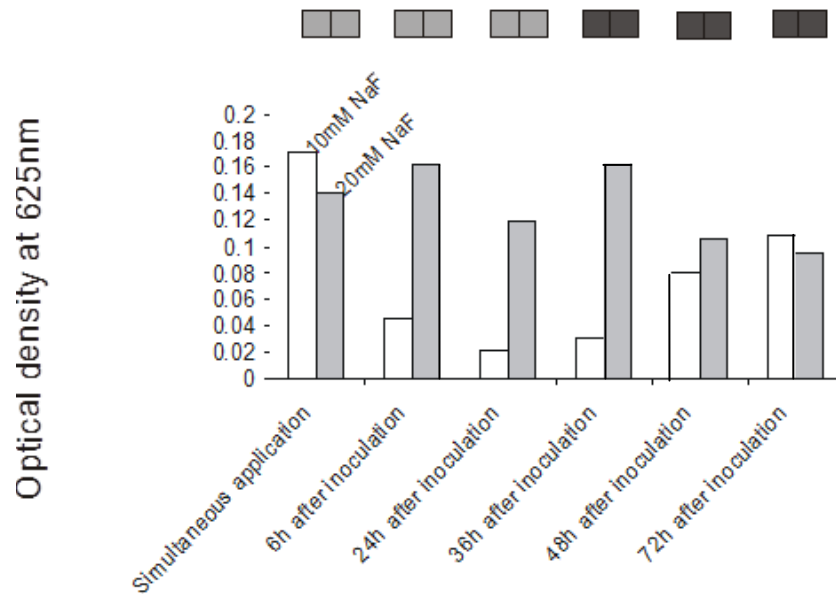


Figure 4. Mean biomass and induced yeasts of *Rhizopus stolonifer* cultivated in synthetic broth incorporated with NaF. No yeast observed after 72 h of growth; terminal budding yeast cells observed after 72 h of growth.

at 6 h application time. When the growth rates for the two application times were compared, that at 72 h application time was higher (Table 6), indicating that the higher rate of growth was as a result of the proliferation of induced yeast cells therein. Therefore, unlike the induced yeast cells, protoplasts were non-proliferating. This probably accounted for the lower growth rate obtained for the simultaneous application where protoplasts were predominant.

Microscopic examination also failed to reveal induced yeast cells in broths with simultaneous application time when 20 mM NaF was incorporated. Figure 4 compares the mean biomass in broths incorporated with 10 and 20 mM NaF at different application times. Although biomass at 10 mM NaF was higher at simultaneous application, curiously its growth rate, $\mu = \log_{10}/h$ (Gibson et al., 1998), was less than that at the 20mM level, as shown in Table 6. The most interesting observation here was that yeast cell was not induced in either media throughout the cultivation period. This indicated that both levels of the inhibitor were equally effective in preventing yeast induction. Except for the simultaneous application time and 72 h application time, the mean biomass at 20 mM NaF was greater than that at the other application times (Figure 4). Figure 4 (upper boxes) also showed the yeast induction possibilities for the 2 different broth media, using 72 h after inoculation as a basis for comparison. Yeast cells were not induced within the first 72 h in the media up till 24 h application time at both 10 and 20 mM levels; but at application time as from 36 h, yeast cells were induced within the period. This indicated that early incorporation of NaF prevented early induction of yeast

cells.

Further examination was done at the 20mM level because of the higher biomass generated as from 6 h application time, as seen in Figure 4. Thus, Figure 5 shows that yeast cell density built up as from 6 h application time, reaching a maximum at 36 h application time and this diminished thereafter, although cell density was still higher than that at 6 and 24 h application times. At 48 h application time and 72 h application time, shrunken and collapsed yeast cells, or their carcasses were observed. This also indicated that the inhibitor, NaF, became death-wise effective on the matured yeast cells.

DISCUSSION

The study of Omoifo (2011a,b) demonstrated that sporangiospores of *R. stolonifer* were transformed to terminal budding yeast cells in synthetic broth. The present results confirmed this. Earlier studies showed that sporangiospores of another Zygomycete, *M. circinelloides*, underwent lateral morphogenetic transformation in media of similar elemental composition (Omoifo, 2006a, b; Omoifo and Awalehmen, 2012). However, our results are different from that of Bartnicki-Garcia and Nickerson (1962b) who cultivated strains of *R. stolonifer* under CO₂ pressure and concluded that unlike *Mucor* species, *R. stolonifer* was not dimorphic. While *M. circinelloides* exhibited sigmoid growth pattern in zinc and myoinositol supplemented broths (Omoifo, 2006b) and K⁺ and Na⁺-modulated broths (Omoifo and Awalehmen, 2012), such regular pattern was not shown

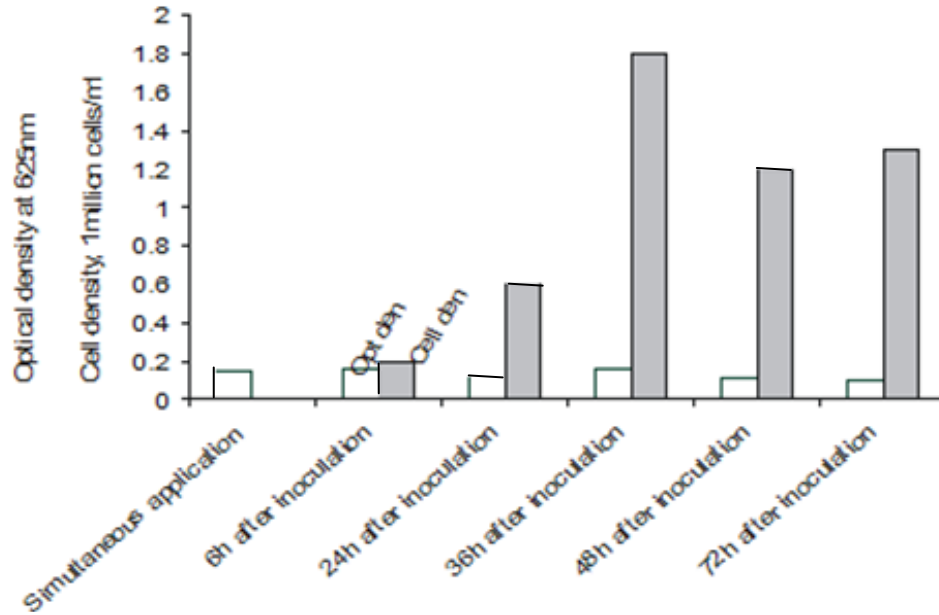


Figure 5. Induced yeast inhibition by 20 mM NaF incorporated into synthetic broth for the cultivation of *Rhizopus stolonifer*. Note: one million sporangiospores were inoculated into each broth-flask and Simultaneous Application time, yeast cells were not found throughout the period of observation.

in the present study, although the aforementioned elements were incorporated in the growth media. Perhaps this was affected by other conditions of growth. The present study was conducted at $28 \pm 1^\circ\text{C}$, while *M. circinelloides* was cultivated at 20°C (Omoifo, 2006a, b; Omoifo and Awalemen, 2012). Several studies have shown that temperature can affect morphological expression of dimorphic fungi, including *Blastomyces dermatitidis* (Levine and Ordal, 1946), *Paracoccidioides brasiliensis* (Edward and Nickerson, 1949) and *Cladosporium werneckii* (Hardcastle and Szaniszló, 1974). Although the growth of *R. stolonifer* in the present study was purely in discrete units in submerged cultures, the temperature of growth could have affected the growth pattern. Furthermore, unlike the study of Omoifo (2011b) where *R. stolonifer* exhibited pH-related sigmoid growth in a Ca^{2+} resplendent medium, Ca^{2+} was not incorporated into the growth medium in the present study. The role of Ca^{2+} in signals transduction circuit, the phosphoinositide cascade, and intracellular ion homeostasis in eukaryotic cells is well known, as it complexes with calmodulin which in turn stimulates many enzymes, ion transport pumps as well as kinases and phosphatases, thus participating in many metabolic and physiological processes, including cell cycle progression in mammals (Kahl and Means, 2003), plants (Hepler, 2005) as well as glycosylation, sorting and protein degradation in yeasts (Durr et al., 1998). Perhaps, it is also required in processes inuring the sigmoid growth pattern during yeast induction from spores of *R. stolonifer*.

The transient morphologies in the sequential conversion

to yeast cells include growth/germ spheres, neoplasts and protoplasts (Omoifo, 2003, 2009, 2011b; Omoifo et al., 2006; Omoifo and Omamor, 2005), and this was confirmed in the present study. Growth spheres were not observed in the control broth; conditions probably existed that facilitated such transition that usually occurred prior to the release of cytosolic nucleates/neoplasts (Omoifo, 2003). Therefore the sequential sporangiospore - yeast transformation (SSYT) hypothesis earlier proposed for such conversion processes (Omoifo, 2003), was presumed to be fundamental to this study. The normal distribution of the morphological entities expressed in this study confirmed the fitness to this hypothesis.

The absence of neoplasts and protoplasts in the control broth proved decisive to this study. That the control broth which induced only terminal budding yeast cells had similar mean biomass with the 5 mM NaF incorporated broth in which the exhibited morphologies were neoplasts, protoplasts and yeast cells, indicated that the inhibitor repressed the conversion of neoplast to protoplast on the one hand and of protoplast to yeast cell on the other. This was probably true of the other levels of NaF where similar transient morphologies were induced. Yet, the mean biomass at 20 mM NaF incorporation was outstanding; the induced morphologies were protoplasts, which predominated and yeast cells. It appeared that at this inhibition level, all neoplastic units converted to protoplasts (note that although internal cell measurements were not obtained in this study, protoplasts had internal dimensions and measurable sizes in contrast to

neoplasts which had extreme sizes and undefined shapes) and these contributed to the high biomass recorded at this level, although they were non-proliferating.

In the second study, when 10 mM NaF was applied at the same time as sporangiospore-inoculum, no yeasts were induced except protoplasts even after 120 h of growth. Similar observation was made at 6 h application time. While the same was true of simultaneous application at 20 mM NaF, the effect of 10 mM NaF at preventing yeast induction was more profound as only the former induced yeast cells, albeit scantily at 6 h application time, and after 120 h of growth. This was in spite of the lower growth rate at 6 h application time. With 10 mM NaF incorporation at 36 h application time, some protoplasts were converted to yeast cells by 72 h of growth. Here, scanty moniliform filaments with apparent septation (Omoifo, 2011a), were part of the morphological expressions. However, if it was realized that at 72 h application time yeast cells had expressed as from 48 h after inoculation, then inhibitor effect was on actively proliferating yeast cells. However, rather than cell density build up with time, there was actual reduction in yeast cell count by 120 h.

Three effects of NaF were deduced from this study. They were (a) complete inhibition of yeast induction (b) delayed induction of yeast form and (c) apoptosis of well-formed and asexually proliferating yeast cells. These events were completely dependent on the time of exposure of the transforming microorganism to the inhibitor. NaF is a well-known inhibitor of the glycolytic process (Voet and Voet, 1995); the key control step in the pathway is the conversion of 2-phosphoglycerate to phosphoenolpyruvate whereby the fluoride ion interacts with the Mg^{2+} -requiring enolase enzyme (Witters and Foley, 1976; Bassetti et al., 2004). Warburg and Christian (1942) were the first workers to show *in vitro* that the inhibitor forms a complex with magnesium ion, which competitively locates at the active site of the enzyme enolase (Cinnasoni, 1972), an effect that is enhanced with high levels of phosphate (Bassetti et al., 2004) thus preventing the execution of dehydration of 2-phosphoglycerate (dehydroxylation at the carbon-3 position) and hence formation of PEP (Voet and Voet, 1995; Christophe et al., 2001). Shearer and Suttie (1970) provided the first direct evidence for fluoride inhibition of enolase in whole (rat liver) cells. They additionally found in their study that although depression of activities of pyruvate kinase also occurred, such effect was attributed to secondary actions of enolase inhibition that caused a change in the concentration of some other metabolites in the glycolytic pathway which they tested, posting negative results therefrom *in vitro*. This result is similar to that of Bucher and Pflerderer (1970) who had earlier shown that NaF failed to inhibit pyruvate kinase *in vitro*. On the other hand, in *Streptococcus salivarius*, a 2.4 mM NaF dose on metabolizing whole cells resulted in decreased

glucose uptake and immediate fall in cellular glucose-6-phosphate content (Kanapka and Hamilton, 1971). York and Van Denmark (1978) obtained copious growth of *Streptococcus mutans* and *Leuconostoc mesenteroides* on glucose and lactose substrates. As these workers found out in their study, the catabolic process of the two substrates, which involve enolase reaction steps, was prevented by NaF but it had no effect on arginine or pyruvate utilization, which excludes enolase activity. Similar results were obtained by Curran et al. (1994) who found that using concentrations beyond 0.001 mM NaF inhibited the enzyme enolase in a quasi-irreversible manner. If we assume the primary and secondary inhibitory effects of NaF on enolase during the conversion of sporangiospores to terminal budding yeast cells in this study, the physiological effect of the enzyme inhibition by NaF would not be limited to the single-step conversion of 2-phosphoglycerate to PEP product, as affectivity of the enzyme could be transcendent. In illustration of this, we referred to the work of Lemaire and Wesolowski-Louvel, (2004) that showed that at the molecular level disruption of the gene that encodes enolase in *Kluyveromyces lactis* caused severe reduction in the transcript levels of the organism's two glucose transporter genes.

Furthermore, transcriptions of hexokinase and pyruvate decarboxylase genes, which encode the enzymes of the glycolytic and fermentative segments respectively, were also impaired. The study of Haarasilta and Oura (1975) showed that in Baker's yeast *S. cerevisiae*, pyruvate carboxylase is expressed maximally under anaerobic fermentation conditions, thus heightening the first step in the production of ethanol from pyruvate, that is, decarboxylation to acetaldehyde and CO_2 . At the protein level in rat hepatocytes, fluoride binding to enolase and phosphoglucomutase inhibit the enzymes' activity while the lowered level of pyruvate kinase observed was in this case attributed to the effect of cAMP which plays a critical role in the phosphorylation of pyruvate kinase (Shahed et al., 1980). Assuming that these activities occurred at the molecular and physiological levels in our study with NaF challenge, then the transient discrete units, neoplasts and protoplasts, both distinct morphologies in the transformation process to the yeast form (Omoifo, 2003, 2006a, b, 2009, 2011a, b; Omoifo and Awalehen, 2012) would probably be unable to sequester energy from the pyruvate kinase step. This was probably reflected on the differing depressive effect on the relative growth rate by the varying levels of NaF tested in this study. If high energy bonds were thus limited, perhaps biosynthetic pathways inducible at and including the latter phase of substrate levels phosphorylation, as well as fermentation could be hampered.

It is necessary to point out that at simultaneous application time at 10 or 20 mM NaF a stable or persistent proliferating morphology, that is, yeast form was never observed except the transient morphologies, neoplasts and protoplasts. In some cultures where proto-

plasts had just been converted to yeast cells, debilitating effect of some levels of NaF caused negative cell growth rate, meaning death ensued, and carcasses left over. This is in sharp contrast to the finding that when protoplasts are transferred to aerobic environment, filamentous growth ensued (Omoifo, 2003). The difference, as we suspect in the present study, was that NaF treatment severely hampered the ability of transforming protoplasts to adopt substrate-level phosphorylation as a process for obtaining high energy bonds in the synthetic broth. While as earlier reported, on transfer to aerobic environment, protoplasts could obtain copious energy supply through oxidative phosphorylation, hence filament formation. This notion was reinforced by the fact that in rapidly growing HeLa cells, NaF insult forces a rapid decrease in cellular ATP content while $^{14}\text{CO}_2$ production level from the TCA cycle activity (oxidative respiration) was unaffected (Carlson and Suttie, 1967). Conversely, glucose uptake in Baker's yeast, *Saccharomyces cerevisiae* D2373-10D, was completely blocked at 50 mM NaF challenge, thus preventing glycolysis; this provided a basis for its (concentration) use in glucose invertase assay as the inhibitor has no effect on the sucrose splitting enzyme, [β -fructofuranosidase, EC 3.2. 1.26] (Silveira et al., 1996), which in *S. cerevisiae* 303-67 may be cell wall-bound, or intracellular (Gascon and Lampen, 1968) just like glycolytic enzymes (Alberts, 1987; Dawes, 1986; Soumalainen and Nurminen, 1973). We point out that this level of concentration (50 mM NaF) inhibited or 10 and 20 mM NaF simultaneous application time completely prevented the induction of terminal budding yeast cells in our present study. Since proliferating yeast cells occurred in control cultures and late phase NaF-application time cultures in the present study, it was not illogical to deduce that the physiological process that led to the induction of the yeast form *R. stolonifer* was partly substrate-level phosphorylation.

Carlson and Suttie (1967) found that NaF challenge on HeLa cells imposed rapid decline in glucose utilization, followed by a slower growth rate. Our study using NaF corroborated the latter claim. In their study, Otsuki et al. (2005) showed that glucose utilization by HeLa cells almost stopped just 4 h after the inhibitor treatment; its action was most profound at 5 to 10 mM NaF. They further showed that NaF treatment induced apoptotic activities thus eliciting enhanced BAD - a pro-apoptotic protein expression, decreased mitochondrial membrane potential, as well as triggered caspase activation, DNA fragmentation and production of apoptotic bodies. In the present study, we found that NaF treatment was inhibitory to yeast induction in the concentration range (5 to 50 mM). Specifically, it caused the collapse of mature yeast cells with conspicuous carcass left-over. These cryptic observations tend to suggest that the metabolically degenerating activities in the NaF- disrupted enolase effects in HeLa cells, as observed by Otsuki and

his colleagues, were involved in the yeast apoptosis in our study. Such cathartics end resulted in the diminished cell count at 48 and 72 h application time with 20 mM NaF incorporation.

Earlier studies showed that the transient morphologies occurred at the pre-logarithmic growth phase (Omoifo and Awalemhen, 2012). Their formation was said to conform to the sequential-sporangiospore-yeast transformation (SSYT) hypothesis (Omoifo, 2003) whereby chemiosmotic transport mechanisms (Albert et al., 1994), also known to control cell volume (Dawes, 1986; Jain, 1972; Bartnicki-Garcia and Lippman, 1977) was thought to regulate sizes of the inoculated spores, converting them to germ spheres (Bartnicki-Garcia and Lippman, 1977) within which simultaneously, as has been argued (Omoifo, 2003) that synchronous DNA replication occurred and this gave rise to cytoplasmic granulation (Omoifo, 2003, 2009). It was assumed that when equilibrium state was reached between the cell interior and the bulk medium, conditions were ripe for activities of lytic enzymes (Maurel and Douzou, 1978). When cell wall lysed, cytosolic neoplasts are exposed to the bulk medium; these subsequently evolve to protoplasts (Omoifo, 2003, 2009). It is thought that a vector ally directed electrogenic ion transport mechanism, as occurs in mitochondria (West and Mitchell, 1972, 1973; Dixon and Hopkin, 1980), in this case initialized by transmembrane-pH-gradient, underlined biochemical and physiological activities leading to structural modifications in the transforming morphologies observed in this study.

The abundance of the terminal budding yeast form in the control cultures in the present study is similar to that reported for *M. circinelloides* Tieghem cultivated in media of similar elemental composition (Omoifo, 2006b). Ionic movements involving K^+ and Na^+ ions, have been recorded during sporangiospore - yeast transformation of *M. circinelloides* (Omoifo and Awalemhen, 2012). It is well known that Na^+ is electrogenically interchanged with K^+ ions through the membrane bound $\text{Na}^+\text{-K}^+$ ATPase (Lingrel and Kuntzweiler, 1994; Tonomura, 1986). Observation showed that commencement of Na^+ ion influx into the intracellular medium coincided with the occurrence of the yeast form (Omoifo and Awalemhen, 2012). In the present study, it was suggested that arising from H^+ -glucose symport, a fact demonstrated for the yeast, *Metschnikowia reukaufii* (Alderman and Hofer, 1981), the fungus, *Neurospora crassa* (Slayman and Slayman, 1974) and arthrosporal fungus, *Geotrichum candidum* (Amrane et al., 1999), the carbon substrate became available in the protoplast interior and subsequently catabolism through glycolysis occurred, using cytoplasm-bound enzymes of glycolysis thus induced as a result of prolonged exposure to the substrate (Merrill and Pitot, 1986; Jansen et al., 2004; Cabib et al., 1988). The absence of yeast cells in NaF-inoculum simultaneous application time - cultures could be reasoned as *ab initio* prevention of induction of enolase dehydrolytic activities

as a result of NaF inhibition of the enzyme even in the possible presence of symported glucose. Assuming this occurred, it could also lead to non-induction of and prevention of activities of beta-glucanase, mannanase and carbon disulphide reductase and other hydrolases, all of which are needed for biosynthesis of the cytoskeletal structure (Soumalainen and Nurminen, 1973; Griffin, 1981; Cabib et al. 1988) that could structurally provide the protoplast the yeast cell wall. The ability of protoplasts of the dimorphic fungus, *Histoplasma capsulatum*, to regenerate the yeast cell form in liquid medium has been demonstrated (Carbonell et al., 1973).

For some experiments, inhibitor incorporation was perhaps done when some early generated neoplasts or protoplasts had progressed beyond critical steps in the yeast induction process, while the effect of the inhibitor was exerted in the majority of protoplasts. If we recognize the transient protoplast as the archithetural format for biophysical structuring of nascent yeast form, that is, cytoskeletal construction which is a high energy demanding process, then this form was the main target for positive impact by NaF. This probably explained the scanty presence of yeast cells in some media. Perhaps still, energy sequestration occurred albeit slowly, even in the presence of inhibitor, bordering on enolase enzyme activation potential. The yeast cells were scanty but observed only at the late phase of cultivation.

Conclusion

This study showed that NaF could prevent the induction of yeast cell, slow down the rate of induction of yeast cell or cause the loss of budding capability and viability and subsequent death of yeast cell induced from sporangiospores of *R. stolonifer* cultivated in synthetic broth. This effect exhibited was dependent on the time of exposure of the microorganism to the glycolytic inhibitor, NaF. This study also showed that sodium fluoride had no effect on protoplast formation, indicating that its (protoplast) induction was not a function of glycolysis, but as previously shown, a function of K⁺ influx into intracellular medium of the transforming microorganism (Omoifo, 2006a; Omoifo and Awalemen, 2012). This study as well as literature referred suggested that the induction of terminal budding yeast cells from sporangiospores of *R. stolonifer* was intimately associated with the glycolytic process. Nevertheless, further studies are needed to examine the contribution of the various inductive enzymes during the transformation process; critical to such evidence would be the genome sequencing for such enzymes.

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REFERENCES

- Albert B, Lewis D, Raff M, Roberts K, Watson JD (1994). The Molecular Biology of Cells, 3rd Edition. New York: Garland Publishing Company, Inc.. p. 1294.
- Alberts BM (1987). Prokaryotic DNA Replication Mechanism. In: Eukaryotic Chromosome Replication, R. A. Laskey, F. R. S. Banks and P. M. Nurse. Eds. London: The Royal Society. pp. 1-26.
- Alderman B, Hofer M (1981). The active transport of monosaccharides by the yeast *Metschnikowia reukaufii*: evidence of an electrochemical gradient of H⁺ across the cell membrane. Exptal Mycol. 5:120-132.
- Amrane A, Plihon F, Prigent Y (1999). Proton transfer in relation to growth of *Geotrichum candidum* and *Penicillium camemberti* in synthetic liquid media. Enzyme Microb. Technol. 24:561-568.
- Bartnicki-Garcia S (1968). Control of dimorphism in *Mucor* by hexoses; inhibition of hyphal morphogenesis. J. Bacteriol. 96:1586-1594.
- Bartnicki-Garcia S, Lippman E (1977). Polarization of cell wall synthesis during spore germination of *Mucor rouxii*. Expt Mycol. 1:230-240.
- Bartnicki-Garcia S, Nickerson WJ (1962a). Induction of yeast - like development in *Mucor* by carbon dioxide. J. Bacteriol. 84: 829- 840.
- Bartnicki-Garcia S, Nickerson WJ (1962b). Nutrition, growth and morphogenesis of *Mucor rouxii*. J. Bacteriol. 84:841-858.
- Bartnicki-Garcia S, Nickerson WJ (1962c). Isolation composition and structure of cell walls of filamentous and yeast-like forms of *Mucor rouxii*. Biochim. Biophys. Acta, 58:102-119.
- Bartnicki-Garcia S, Nickerson WJ (1962d). Assimilation of carbon dioxide and morphogenesis of *Mucor rouxii*. Biochim Biophys. Acta 64:548-551.
- Basseti WHC, Ming-Ho Y, Struas S (2004). Environmental Toxicology. CRC Press. p. 368
- Bucher T, Pfeleiderer GP (1970). Pyruvate kinase from muscle. In: Methods in Enzymology, Colowick SP, Kaplan NO, Eds. New York: Academic Press, p. 435.
- Cabib E, Bowers B, Sburlati A, Silverman SJ (1988). Fungal cell wall synthesis: the construction of a biological system. Microbiol. Sci. 5: 370-375.
- Carbonell FG, Gil F, Yegres F (1973). Cell wall regeneration of protoplasts of *Histoplasma capsulatu*. In: Yeasts, Mould and Plant Protoplasts. Manueva JRU, Garcia-Acha I, Gascon S, Uruburu F, Eds. New York: Academy Press, p. 93-103.
- Carlson JR, Suttie JW (1967). Effect of sodium fluoride on HeLa cells: 11. Metabolism alterations associated with growth inhibition. Exptal Res. 45(2): 423-432.
- Christophe LM, Verlinde J, Hennaert V, Blonski C, Willson M, Perie JJ, Gelb MH, Hol WGJ, Michels PAM (2001). Glycolysis as a target for the design of new anti-trypanosome drugs. Drug Resist. Updates, 4:1-14.
- Cinnasoni G (1972). The inhibition of enolase by fluoride *in vitro*. Caries Res. 6: 93-102.
- Curran TM, Buckley DH, Marquis RE (1994). Quasi-irreversible inhibition of enolase of *Streptococcus mutans* by fluoride. FEMS Microbiol. Lett. 119(3): 283-288.
- Dawes EA (1986). Microbial Energetics: Tertiary Level Biology. New York: Chapman and Hall, p. 189.
- Dixon JF, Hopkin LE (1980). The reconstituted (Na,K⁺)-ATPase is electrogenic. J. Biol. Chem. 255:10681-10686.
- Durr G, Strayle J, Plemper R, Elbs S, Klee SK, Catty P, Wolf DH, Rudolph HK (1998). The medial-golgi ion pump pmr1 supplies the yeast secretory pathway with Ca²⁺ and Mn²⁺ required for glycosylation, sorting and endoplasmic reticulum-associated protein degradation. Mol. Biol. Cell, 9:1149-1162.
- Edward GA, Nickerson WJ (1949). Studies on the physiological bases of morphogenesis in fungi. The respiratory metabolism of dimorphic fungi. J. Gen. Physiol. 33: 41-55.
- Fisher JJ (1977). Induction of yeastlike growth in Mucorales by systemic fungicides and other compounds. Trans. Brit. Mycol. Soc. 68: 397-402.

- Gascon S, Lampen JO (1968). Purification of the invertase of yeast. *J. Biol. Chem.* 243(7):1567-1572.
- Gibson AM, Bratchell N, Roberts TA (1988). Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. *Internatl. J. Food Microbiol.* 6:155-178.
- Griffin DH (1981). *Microbial Physiology*, John Wiley and Sons. New York. 383p.
- Haarasilta S, Oura E (1975). Effect of aeration on the activity of gluconeogenic enzymes in *Saccharomyces cerevisiae* growing under glucose limitation. *Archiv. Microbiol.* 31:106(3):271-3.
- Hall MJ, Kolankaya N (1974). The Physiology of mould, yeast dimorphism in the genus *Mycotypha* (Mucorales), *J. Gen. Microbiol.* 82:25-34.
- Hardcastle RV, Szanislo PJ (1974). Characterization of dimorphism in *Cladosporium werneckii*. *J. Bacteriol.* 119:294-301.
- Hepler PK (2005). Calcium: A Central Regulator of Plant Growth and Development. *Plant Cell* 17:2142-2155.
- Jain MK (1972). *The Biomolecular Lipid Membrane: A System*, New York: Van Nostrand Reinhold Company, p. 467.
- Jansen MLA, Daran-Lapujade P, deWinde JH, Piper MDW, Pronk JY (2004). Prolonged maltose-limited cultivation of *Saccharomyces cerevisiae* selects for cells with improved maltose affinity and hypersensitivity. *Appl. Environ. Microbiol.* 70(4): 1956-1963.
- Kahl CR, Means AR (2003). Regulation of cell cycle progression by calcium/calmodulin-dependent pathways. *Endocrine Rev.* 24(6):719-736.
- Kanapka JA, Hamilton IR (1971). Fluoride inhibition of enolase activity in vivo and its relationship to the inhibition of glucose-6-P formation in *Streptococcus salivarius*. *Arch. Biochem. Biophys.* 14(1):167-174.
- Lemaire M, Wesolowski-Louvel M (2004). Enolase and glycolytic flux play a role in the regulation of the glucose permease gene, RAG1 of *Kluyveromyces fragilis*. *Genetics* 168: 723-731.
- Levine S, Ordal ZJ (1946). Factors influencing the morphology of *Blastomyces dermatitidis*. *J. Bacteriol.* 5: 687 -694.
- Lingrel JB, Kuntzweiler T (1994). Na⁺, K⁺-ATPase. *J. Biol. Chem.*, 259: 19659-19662.
- Lubberhusen TL, Nielsen T, McIntyre M (2003). Characterization of the *Mucor circinelloides* life cycle by on-line image analysis. *J. Appl. Microbiol.* 95: 1152-1160.
- Maurel P, Douzou P (1978). Cation-induced regulatory mechanism of enzyme reaction. In: B, Pullman Ed. *Frontiers in Physicochemical Biology*, New York: Academic Press, pp. 421-457.
- McIntyre M, Breum J, Arnau J, Nielsen J (2002). Growth physiology and dimorphism of *Mucor circinelloides* (synracemosus) during submerged batch cultivation. *Appl. Microbiol. Biotechnol.* 58: 495-502.
- Merrill MJ, Pitot HC (1986). The use of tissue culture techniques in the study of gluconeogenesis. In: *Hormonal Control of Gluconeogenesis*. N. Kraus-Friedmann Ed. Boca/Raton, Florida: CRC Press. pp. 121-127.
- Mooney DT, Sypherd PS (1976). Volatile factor involved in the dimorphism of *Mucor racemosus*. *J. Bacteriol.* 126: 1266-1270.
- Mysyakina IS, Funtikova NS (2000). Lipid composition of the yeastlike and mycelial *Mucor hiemalis* cells grown in the presence of 4-chloroaniline. *Microbiology* 69:670-575.
- Omoifo CO (2003). Sequential sporangiospore-yeast transformation hypothesis. *Afric. Scientist*, 4:191-273.
- Omoifo CO (2006a). Effect of K⁺, Na⁺ and uracil on sporangiospore-yeast transformation of *Mucor circinelloides* Tiegh. *Afric. J. Biotechnol.* 5(9): 715-722.
- Omoifo CO (2006b). Effect of myoinositol and zinc on sporangiospore-yeast transformation of *Mucor circinelloides* Tiegh. *Afric. J. Biotechnol.* 5(9):723-730.
- Omoifo CO (2011a). *Rhizopus stolonifer* exhibits dimorphism. *Afric. J. Biotechnol.* 10(20):4269-4275.
- Omoifo CO (2011b). Effect of extracellular calcium chloride on sporangiospore-yeast transformation of *Rhizopus stolonifer*. *Afric. J. Biotech.* 10(20):4276-4288.
- Omoifo CO, Awalemben BI (2012) Sporangiospore-yeast transformation of *Mucor circinelloides*: ionic circulation, chemical potential, sodium influx rate and morphogenesis. *Afric. J. Biotechnol.* 11(14):3217-3442.
- Omoifo CO, Aruma MB, Omamor IB (2006). Effect of myoinositol on sporangiospore-yeast transformation of *Mucor circinelloides* Tieghem cultivated in synthetic broth. *Hind. Antibiot. Bull.* 48: 24-31.
- Omoifo CO, Omamor IB (2005). Effect of zinc on sporangiospore-yeast transformation of *Mucor circinelloides* Tiegh. *ASSET Series B*, 4(1):159-166.
- Otsuki S, Morshed SR, Chowdhury SA, Takayama F, Satoh T, Hashimoto K, Sugiyama K, Amano O, Yasui T, Yokote Y, Akahane K, Sakagami H (2005). Possible link between glycolysis and apoptosis induced by sodium fluoride. *J. Dent. Res.* 84(10):919-923.
- Price JS, Storck R, Gleason FH (1973). Dimorphism of *Cockeroomyces potrasii* and *Mycotypha microspora*. *Mycologia* 65:1275-1283.
- Rogers PJ, Clark - Walker GD, Stewart PR (1974). Effect of oxygen and glucose on energy metabolism and dimorphism of *Mucor genesisis* grown in continuous culture: Reversibility of yeast mycelium conversion, *J. Bacteriol.* 119:282-293.
- Romano AH (1966). Dimorphism In: G. C Ainsworth, A. S Sussman, Eds. *The Fungi: An Advanced Treatise*. New York: Academic Press, 2:181-202.
- Schulz BE, Kraepelin G, Hinkelmann W (1974). Factors affecting dimorphism in *Mycophyta* (Mucorales): a correlation with the fermentation/respiration equilibrium. *J. Gen. Microbiol.* 83:1-13.
- Shahed AR, Miller A, Allmann DW (1980). Effect of fluorine containing compounds on the activity of glycolytic enzymes in rat hepatocytes. *Biochem. Biophys. Comm.* 94(3):901-908.
- Sharma VC, Ayanru DKG, Ogebe ON (1984). Maximization of fuel ethanol from pawpaw fermentation. *Energy* 9:15-20.
- Shearer TR, Suttie JW (1970). Effect of fluoride on glycolytic and citric acid cycle metabolites in rat liver. *J. Nutri.* 100:749-756.
- Silveira MCF, Carvajal E, Bon EPS (1996). Assay for in vivo yeast invertase activity using NaF. *Anal. Biochem.* 238:26-29.
- Slayman CL, Slayman CW (1974). Depolarization of the plasma membrane of *Neurospora* during active transport of glucose: Evidence for a proton-dependent cotransport system. *Proc. Nat. Acad. Sci. USA.* 71:1935-1939.
- Soumalainen H, Nurminen T (1973). Structure and Function of the Yeast Cell Envelope. In: *Yeast, Mould and Plant Protoplasts*, Manueva JR, Garcia-Acha G, Uruburu F, Eds. New York: Academic Press. pp. 93-103.
- Tonomura Y (1986). Na⁺, K⁺-ATPase in the plasma membrane. In: *Energy-transducing ATPase- Structure and Kinetics*. London: Cambridge University Press, pp. 240-281.
- Voet D, Voet JG (1995). *Biochemistry* 2nd edn. New York: John Wiley and Sons Inc., 1390p.
- Warburg O, Christian W (1942). Chemical mechanism of fluoride inhibition of yeast. *Naturwissenschaften* 29:590-595.
- West JC, Mitchell P (1972). Proton-coupled S.galactoside translocation in non-metabolizing *Escherichia coli*. *J. Bioenergetics*, 3: 445-463.
- West JC, Mitchell P (1973). Stoichiometry of lactose-proton symport across plasma membrane of *Escherichia coli*. *Biochem. J.* 132:587-592.
- Witters WL, Foley CW (1976). Effect of selected inhibitors and methylene-blue on possible phosphogluconate pathway in washed boar sperm. *J. Anim Sci.* 43: 159-163.
- Yamaguchi H (1975). Control of dimorphism in *Candida albicans* by zinc: effect on cell morphology and composition. *J. Gen. Microbiol.* 86: 370-372.
- York KG, van Demark PJ (1978). Growth inhibition of *Streptococcus mutans* and *Leuconostoc mesenteroides* by sodium fluoride and ionic tin. *Appl. Environ. Microbiol.* 35 (5):920-924.