# Physico-chemical studies on the growth of an Ochratoxin A-degrading *Rhizopus* sp.

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**Abstract:**Studies were conducted on the effects of some physico-chemical factors such as pH, temperature and various mineral elements on the vegetative growth of an Ochratoxin A-degrading *Rhizopus* sp. The pH studies revealed an increase in mycelial weight as the pH of the medium increase, with a mycelial weight of $18.0 \pm 2.0333$ mg/50cm$^3$ at pH 3.0, $73.0 \pm 1.0837$ mg/50cm$^3$ at pH 4.0 before reaching the peak ($106.0 \pm 1.4204$ mg/50 cm$^3$) at pH 5.0. Thereafter, an increase in pH resulted in a reduction in mycelia weight with no growth detected at pH 9.0. Temperature studied showed that a temperature of 30 ℃ produced the highest mycelial weight of 145.5 mg/50 cm$^3$ closely followed by 120.6 mg/50 cm$^3$ at 35 ℃ while the lowest mycelial weight (15.5 mg/50 cm$^3$) was recorded at 20 ℃ and no growth detected at 15 ℃. Among the various microelements investigated, Zinc appears to be the most important with a Zinc-free medium producing the poorest mycelia weight of $37.01 \pm 0.6666$ mg/ 50 cm$^3$ while Cobalt appears not to play any significant role in the growth of this *Rhizopus* sp. as the Cobalt-free medium produced identical mycelial weight (57.3 mg/50 cm$^3$) with the medium containing all the nutrients. Investigation of the macroelement requirement of the organism showed that Calcium is the most important with a Calcium-free medium producing the poorest growth of $15.0 \pm 0.7676$ mg/50 cm3 while the best growth was ($50.7 \pm 0.4096$ mg/50 cm$^3$) was observed in a complete medium without Magnesium.
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## Introduction

Mycotoxins are chemically and structurally diverse compounds produced by many fungi as their secondary metabolites which under certain environmental conditions can contaminate food and feed ingredients. These mycotoxins usually contaminate food and feed ingredients in two ways: the fungi may grow as pathogens on plants crops or may grow saprophytically on stored plant products (Gleen, 2007) and the ingestion of these mycotoxins often results in undesirable biological reactions varying from acute disease state and death, to chronic disease states, and economically important but clinically obscure changes in growth, production and immunosuppression (Bakutis *et al*., 2005), reduced reproductive capacities (Fink-Gremmels, 2005).

Among the vast majority of mycotoxins identified, Ochratoxin A (OTA) is one of the most important (Abrunhosa *et al*., 2010) and it has been experimentally shown to be teratogenic, a potent renal carcinogen, immunosuppressive, an enzyme inhibitor with effects on lipid peroxidation. It has also been listed as a possible carcinogen of group 2B by the International Agency for Research on Cancer (IARC, 1995). It has also been implicated in Balkan Endemic Nephropathy (BEN) (Pfohl-Leszkowicz *et al*., 2002).

Owing to the adverse health conditions (in some cases death) and several economic losses resulting from consumption of Ochratoxin A-contaminated food and feed commodities, various pre- and post-harvest strategies have been identified to prevent or at least reduce to the barest minimum the adverse effects resulting from exposure of man and animal to mycotoxins (Jouany, 2007). The post harvest strategies are usually categorised into three: physical, chemical and biological, however, the most promising approach to decontaminate feed is the biological detoxification, since enzymes are substrate specific (Liu *et al*., 2001).

Several attempts into the biological detoxification of Ochratoxin A have led to the publication of several reports involving the use of different organisms to degrade OTA (Sÿkrinjar *et al*., 1996; Varga *et al*., 2000; Abrunhosa *et al*., 2002; Varga *et al*., 2005; Fuchs *et al*., 2008; Abrunhosa *et al*., 2010; Mateo *et al*., 2010). However, there is paucity of information on the nutrition requirements of these Ochratoxin A-degrading organisms which is important when considering the use of these organisms in biological detoxification of Ochratoxin A. This study attempts were made to investigate the effect physic-chemical parameters on the growth of a *Rhizopus* sp. that is capable of degrading about 90%

of 8.0 mg Ochratoxin A per litre in vivo after 15 days of incubation at 30 °C (Garuba, data unpublished).

## Materials and methods
## Microorganism

A recently isolated *Rhizopus* sp. from spoilt "Ori" (a Nigerian fermented food) capable of degrading about 90% of 8.0 mg Ochratoxin A per litre in vivo after 15 days of incubation at 30 °C (Garuba, data unpublished). was used in this study. The organism was maintained on Potato Dextrose Agar slants supplemented with chloramphenicol 50 ppm at 4 °C.

## Inoculum preparation

Inoculum used in this study was prepared using the method of Nahar *et al*. (2008).

## Effect of pH

The effect of varying temperature regimes on the mycelia growth of *Rhizopus* sp. was determined in a basal containing (g/l) $FeSO_4$,(0.01), $MgSO_4$. $7H_2O$ (0.5), $KH_2PO_4$ (0.05), fructose (10), yeast extract (2.5), $KNO_3$ (1.55). The medium was adjusted pH values of 3, 4, 5, 6, 7, 8, and 9 using 0.1 M HCl or 0.1 M NaOH and then dispensed in 50 ml aliquots into 250 ml Erlenmeyer Flasks and sterilized by autoclaving at 121 °C for 15 minutes. Each flask was then separately inoculated with a 5 mm mycelia disc of a 5 day old *Rhizopus* sp. and incubated for 120 h at 30 °C. The mycelia was harvested and quantified using the mycelia dry weight as described by Fasidi and Olorunmaiye (1994). Each treatment was done in triplicates.

## Temperature

The effect of varying temperature regimes on the mycelia growth of *Rhizopus* sp. was determined using the basal medium above. The medium was adjusted to the optimum growth pH and dispensed in 50 ml aliquot into 250 ml Erlenmeyer flasks, sterilized and inoculated as described above. Incubation was carried out at 20 °C, 25 °C, 30 °C, 35 °C, 40 °C and 45 °C respectively for 120 h and each treatment was set up in triplicates. After 120 h of incubation, the mycelia was harvested and the mycelia dry weight was determined as previously described.

## Effect of different Mineral elements
*Microelement*

The basal medium of Fasidi and Jonathan (1994) was employed for this study with the sulphate form of the trace elements ($Fe^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Co^{2+}$, $Cu^{2+}$) supplemented (10.0 mg/L) separately in the basal medium. Controls with all and without any trace element were also set up

*Macroelement*

The basal medium used for the microelements above was also employed for this study. However, $CaCl_2$ was replaced with $NH_4Cl$ in the medium to study the effect of Calcium and also $KH_2PO_4$, $MgSO_4$, $NaNO_3$ replaced with their corresponding ammonium conjugate to study the effect of Potassium, Magnesium and Sodium respectively. Controls were also set up as previously described above.

## Statistical analysis

Results obtained in this study were subjected to analysis of variance using ANOVA and separation of means was carried out by Duncan's Multiple Range Test (Duncan, 1955).

## Results and Discussion

The results of effect of different pH regimes (pH 3-9) on the growth of this *Rhizopus* sp. are presented in Table 1. The results showed that the organism is capable of growing over a wide range of pH, however, the best mycelial growth of $106.0\pm1.4204$ mg/50 $cm^3$ was obtained at slightly acidic pH (5.0) which was closely followed and significantly different ($P \leq 0.01$) from $98.0\pm0.8505$ mg/50 $cm^3$ at pH 6.0. The results also indicated poor mycelial growth at pH 8 ($45.1\pm0.3712$ mg/50 $cm^3$) and no growth was observed at pH 9 (Table 1). These results agree with that of Dix and Webster (1995) which reported that fungi naturally grow at acidic pH. Similarly, Owens *et al*. (2002) using the hyphal extension method reported an optimal growth of *Rhizopus* sp. at slightly acidic pH. This *Rhizopus* sp. can then be said to be acidophilic. This observation could be due to the fact that at the optimum pH (5.0), the permeability of the cell wall reaches its optimum allowing the easy diffusion of nutrients needed for growth into the cell (Griffin, 1994).

The influence of different temperature regimes on the growth of the *Rhizopus* sp. showed that all the temperature tested (except 15 °C) supported the growth of this organism. The highest mycelial weight (145.5 mg/50 $cm^3$) was however recorded at 30 °C closely followed by 120.6 mg/50 $cm^3$ at 35 °C and 80.6 mg/50 $cm^3$ at 25 °C. Thus the optimum temperature for this *Rhizopus* sp. was 30 °C. The poorest mycelial growth of 15.5 mg/50 $cm^3$ was however recorded at 20 °C with no growth detected at 15 °C. Similar results were also observed by Nahas (1988) which reported a growth temperature range of 20-40 °C for *Rhizopus oligosporus* and Huang *et al*. (2003) which reported a temperature growth range of 30-38 °C for the growth of *Rhizopus arrhizus*. Furthermore, Kungus (2011) and Zhang *et al*. (2007) reported that temperature is one of the most important

environmental factors affecting the fungal growth and metabolite production hence it is important to know the optimum temperature for growth of each microorganism. The optimum growth temperature with the highest mycelia weight could be adduced to the fact this temperature is favourable for the efficient progression of chemical reactions necessary for growth of the organism (Burge, 2006).

The effects of various micro and macro elements on the vegetative growth of this *Rhizopus* sp. is presented in Table 3. The results revealed that certain mineral elements are needed for growth by the *Rhizopus* sp than others. A complete medium without $Zn^{2+}$ gave the poorest growth of $37.01\pm0.6666$ mg/50 $cm^3$ closely followed and significantly ($P \leq 0.01$) different by a complete medium without $Fe^{2+}$ with a mycelial weight of $43.01\pm0.6667$ mg/50 $cm^3$ and the $Mn^{2+}$-free medium ($50.0\pm0.3333$ mg/50 $cm^3$). Zinc has been reported to be an essential component of various enzyme systems involved in energy production, protein synthesis, and growth regulation (Moat *et al.*, 2002; Prescott *et al.*, 2008), hence confirming the poor growth observed in a Zinc-free medium in this study. The poor growth observed with the Iron-free medium could be as a result of the improper functioning of a heme-like porphyrin and iron-sulfur cluster which are needed for electron transfer needed for growth (Moat *et al.*, 2002). Poor growth observed in the $Mn^{2+}$-free medium could be due to improper functioning of phosphate group transfer system during cellular respiration (Prescott *et al.*, 2008). A complete medium without $Co^{2+}$ gave mycelial weight of 57.3 mg/50 $cm^3$, value of which is similar to value observed in the medium containing all the nutrients ($57.0\pm1.6607$ mg/50 $cm^3$). This suggests that the incorporation of this element into the medium does may not play a significant role in the growth of this *Rhizopus* sp.

Table 1: Effect of pH on the vegetative growth of *Rhizopus* sp.

| pH | Mycelial dry weight (mg/50 $cm^3$) |
|---|---|
| 3 | $18.0\pm2.0333^a$ |
| 4 | $73.0\pm1.0837^b$ |
| 5 | $106.0+1.4240^{ab}$ |
| 6 | $98.0\pm0.8505^{abc}$ |
| 7 | $66.0\pm1.8037^{ab}$ |
| 8 | $45.1\pm0.3712^c$ |
| 9 | - |

Data are means of three replicates $\pm$ SEM. Values followed by the same letters are not significantly different by Duncan's multiple range test ($P \leq 0.01$).

Table 2: Effect of different temperature regimes on the vegetative growth of *Rhizopus* sp.

| Temperature (ºC) | Mycelia dry weight (mg/50 $cm^3$) |
|---|---|
| 15 | - |
| 20 | $15.5\pm0.8821^a$ |
| 25 | $80.6\pm0.1155^b$ |
| 30 | $145.5\pm0.0000^a$ |
| 35 | $120.6\pm0.5812^e$ |
| 40 | $34.6.5\pm0.0000^a$ |
| 45 | $20.8\pm0.6667^c$ |

Data are means of three replicates $\pm$ SEM. Values followed by the same letters are not significantly different by Duncan's multiple range test ($P \leq 0.01$).

Table 3: Effect of mineral elements on the vegetative growth of *Rhizopus* sp.

| Mineral elements | Mycelia dry weight (mg/50 $cm^3$) |
|---|---|
| Microelements | |
| Basal medium only (control 1) | $18.3\pm0.2796^{ab}$ |
| Complete medium minus $Co^{2+}$ | $57.3\pm0.000^{cd}$ |
| Complete medium minus $Cu^{2+}$ | $45.00\pm0.6667^c$ |
| Complete medium minus $Fe^{2+}$ | $43.01+0.6667^{ab}$ |
| Complete medium minus $Mn^{2+}$ | $50.01\pm0.3333^a$ |
| Complete medium minus $Zn^{2+}$ | $37.01\pm0.6666^{ab}$ |
| Complete medium with all the elements (control 2) | $57.0\pm1.6607^c$ |
| Macroelements | |
| Basal medium only (control 1) | $15.0\pm2.8868^b$ |
| Complete medium minus $Ca^{2+}$ | $15.0\pm0.7676^a$ |
| Complete medium minus $K^+$ | $48.6\pm1.4530^a$ |
| Complete medium minus $Mg^{2+}$ | $50.7\pm0.4096^b$ |
| Complete medium minus $Na^+$ | $33.3\pm0.6667^{ab}$ |
| Complete medium + all elements | $53.30\pm7.264^{bc}$ |

Data are means of three replicates $\pm$ SEM. Values followed by the same letters are not significantly different by Duncan's multiple range test ($P = 0.01$)

In the series of macroelements investigated in this study, a complete medium without calcium gave the poorest growth ($15.0\pm0.7676$ mg/50 $cm^3$), followed by a complete medium without sodium and potassium with mycelial weights of $33.3\pm0.6667$ mg/50 $cm^3$ and $48.6\pm1.4530$ mg/50 $cm^3$ respectively while a complete medium without magnesium had the best growth ($50.7+0.4096$ mg/50 $cm^3$) (Table 3). This result implies that all the macroelements investigated in this study play significant role in the growth of this *Rhizopus* sp. with calcium being the element probable needed most. Calcium has been reported to play a significant role in cell regulation, maintenance of cell structure and cell differentiation

process (Dominguez, 2004), hence, a poor growth observed in the calcium free medium. Poor growth recorded in the potassium-free medium could be a as a result of improper regulation of cellular osmotic potential which brings about turgor pressure needed for fungal growth (Jonathan and Fasidi, 2003). Magnesium is reported to act as cofactor for many enzymes, complexes with ATP and also stabilizes ribosome and cell membrane (28) confirming the poor growth observed in the Magnesium-free medium.

In conclusion, this paper reports that for the cultivation of this Ochratoxin A-degrading *Rhizopus* sp. on synthetic media, a pH 5.0, temperature of 30 ℃ will be appropriate. Also various mineral elements such as Calcium, Magnesium, Sodium and Potassium (among the macroelements) and Manganese, Zinc, and Iron (among the microelements) needed to be included in synthetic medium for optimum growth of the *Rhizopus* sp. Information such as this can be employed in the use of this organism for decontamination and detoxification of OTA-contaminated food and feed commodities thereby, reducing the problems associated with the exposure of humans and animals to OTA-contaminated food and feed commodities in these areas.

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## References

1. Abrunhosa, L., Robert R., Paterson, M. and Venâncio, A. Biodegradation of Ochratoxin A for food and feed decontamination. *Toxins* 2010; 2: 1078-1099.
2. Abrunhosa, L., Serra, R. and Venancio, A. Biodegradation of Ochratoxin A by Fungi isolated from Grapes. Journal of Agriculture and Food chemistry 2002; 50:7493-7496.
3. Bakutis, B., Baliukoniene, V. and Paskevicius, A. 2005. Use of Biological Method For Detoxification Of Mycotoxins. Botanica Lithuanica, Suppl**.** 2005; **7**: 123–129.
4. Burge, H. How Does Heat Affect Fungi? The Environmental Reporter 2006; 4(3): 1-3.
5. Dix, N. J. and Webster, J. Fungal ecology. London: Chapman & Hall, 1995.
6. Dominguez, D. C. Calcium signalling in Bacteria. Molecular Microbiology 2004; 54(2): 291-297.
7. Duncan, D B. Multiple range and multiple F tests. Biometrics 1955; 11:1–42,.
8. Fasidi, I. O. and Jonathan, S. G. Growth requirements of V.esculenta (Mass) Singer, a Nigerian Edible Mushroom. Chem. Mikrobiol. Technol. Lebensm 1994; 16:5/6.151-155.
9. Fasidi, I. O. and Olorunmaiye, K. S. Studies on the requirements for vegetative growth of *Pleurotus tuber-reqium* (Fr.) Singer a Nigerian Mushroom. Journal of Food Chemistry 1994; 50: 397-401.
10. Fink-Gremmels, J. Conclusions from the workshops on ochratoxin A in food: recent developments and significance, organized by ILSI Europe in Baden (Austria). Food Additives Contaminants 2005; 22 (Suppl. 1), 1–5.
11. Fuchs, S., Sontag, G., Stidl, R., Ehrlich, V., Kundi, M. and Knasmuller, S. Detoxification of patulin and ochratoxin A, two abundant mycotoxins, by lactic acid bacteria. Food and Chemical Toxicology 2008 46:1398–1407.
12. Glenn, A.E. Mycotoxigenic *Fusarium* species in animal feed. In: Morgavi, D.P., Riley, R.T. (Eds.), *Fusarium* and their toxins: Mycology, occurrence, toxicity, control and economic impact. Animal and Feed Science Technology, 2007.
13. Grffin, D. H. Fungal Physiology (2nd Ed) New York. Wiley Liss. 1994.
14. Huang, L. P., Jin, B., Lant, P and Zhou, J. Biotechnological production of lactic acid integrated with potato wastewater treatment by *Rhizopus arrhizus*, Journal of Chemical Technology and Biotechnology 2003; 78: 899–906.
15. IARC (International Agency for Research on Cancer) Ochratoxin A. IARC. Monogr. Eval. Carcinog. Risks Hum.: Some Naturally Occurring Substances, Food Items and Constituents, Aromatic Amines and Mycotoxins 1993; 56: 26–32.
16. Jonathan, S. O. and Fasidi I.O. Requirements for vegetative growth of *Tricholoma lobayensis* (Heim), a Nigerian edible fungus. Advances in Food Science 2003; 25(3): 91-95.
17. Jouany, J. P. Methods for preventing, decontaminating and minimizing the toxicity of mycotoxins in feeds. Animal Feed Science and Technology 2007; 137: 342-362.

18. Kungus, J. Factors that affect the growth of moulds http://www.moldbacteria.com. Accessed on 10-01-2011.

19. Liu, D.-L., Yao, D. S., Liang, Y.-Q., Zhou, T.-H., Song, Y.-P., Zhao, L. and Ma, L. Production, purification and characterization of an intracellular aflatoxin-detoxifizyme from *Armillariella tabescens (E-20)*. Food and Chemical Toxicology 2001; 39: 461-466.

20. Mateo, E. M., Medina, A., Mateo, F., Valle-Algarra, F. M., Pardo, I. And M. Jiménez. Ochratoxin A removal in synthetic media by living and heat-inactivated cells of *Oenococcus oeni* isolated from wines. Journal of Food Contamination 2010; 21, 23–28.

21. Moat, A. G., Foster. J. W. and Spector, M. P. Microbial Physiology. 4th edition. John Wiley and Sons. 2002.

22. Naha, E. Control of Lipase production by Rhizopus oligosporus under various growth conditions. Journal of General Microbiology 1988; 134: 227-233.

23. Nahar, S., Hossain, F., Feroza, B. And M. A. Halim. Production of glucoamylase by *Rhizopus* sp. in liquid culture. Pakistan Journal of Botany 2008, 40(4): 1693-1698.

24. Owens, J. D., Westbby, A., Kendall, M. and Spurringa, R. A. effect of temperature, pH, water activity and $CO_2$ concentration on growth of Rhizopus oligosporus NRRL 2710. Journal of Applied Microbiology 2002; 92 (2):329-337.

25. Pfohl-Leszkowicz, A., Petkova-Bocharova, T., Chernozemsky, I.N., Castegnaro, M. Balkan endemic nephropathy and associated urinary tract tumours: a review on aetiological causes and the potential role of mycotoxins. Food Additives and Contaminants. 2002; 19 (3):282–302.

26. Prescott, L. M. Harley, J. P. and Klein, D. A. Microbiology, (7th Ed) McGraw-Hill companies, New York. 2008.

27. Sÿkrinjar, M.; Rasic, J. L. and Stojicic, V. Lowering of ochratoxin A level in milk by Yoghurt bacteria and bifidobacteria. Folia Microbiol 1996; 1: 26-2.

28. Varga, J., Peteri, Z., Tabori, K., Teren, J., and Vagvolgyi, C. Degradation of ochratoxin A and other mycotoxins by Rhizopus isolates. International Journal of Food Microbiology. 2005; 99: 321– 328.

29. Varga, J., Rigo, K. and Teren, J. Degradation of ochratoxin A by *Aspergillus* species. International Journal of Food Microbiology. 2000; 59: 1-7.

30. Zhang, Z. Y., Jina, B. and Kelly, J. M. Production of lactic acid from renewable materials by *Rhizopus* fungi. 2007; Biochemical and Engineering Journal 35: 251–263.

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