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

Ilesanmi Fadahunsi 1 Emmanuel Garuba 2 and Olayinka Elutade 2

1Department of Microbiology, University of Ibadan, Ibadan, Nigeria
2Department of Biological sciences Bowen University, Iwo, Nigeria

oluwaseungaruba@live.com, samifadahunsi@yahoo.com

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+2.0333 mg/50 cm 3 at pH 3.0, 73.0±1.0837 mg/50 cm 3 at pH 4.0 before reaching the peak (106.0±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 °C produced the highest mycelial weight of 145.5 mg/50 cm 3 closely followed by 120.6 mg/50 cm 3 at 35 °C while the lowest mycelial weight (15.5 mg/50 cm 3) was recorded at 20 °C and no growth detected at 15 °C. 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±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±0.7676 mg/50 cm 3 while the best growth was (50.7±0.4096 mg/50 cm 3) was observed in a complete medium without Magnesium.

Keywords: Temperature, pH, Microelements, Macroelements, physic-chemical

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 (Šinkyrije 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) FeSO₄(0.01), MgSO₄·7H₂O (0.5), KH₂PO₄ (0.05), fructose (10), yeast extract (2.5), KNO₃ (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 (Fe²⁺, Mn²⁺, Zn²⁺, Co²⁺, Cu²⁺) 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, CaCl₂ was replaced with NH₄Cl in the medium to study the effect of Calcium and also KH₂PO₄, MgSO₄, NaNO₃ 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±1.4204 mg/50 cm³ was obtained at slightly acidic pH (5.0) which was closely followed and significantly different (P < 0.01) from 98.0±0.8505 mg/50 cm³ at pH 6.0. The results also indicated poor mycelial growth at pH 8 (45.1±0.3712 mg/50 cm³) 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 cm³) was however recorded at 30 °C closely followed by 120.6 mg/50 cm³ at 35 °C and 80.6 mg/50 cm³ at 25 °C. Thus the optimum temperature for this Rhizopus sp. was 30 °C. The poorest mycelial growth of 15.5 mg/50 cm³ 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 added 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 $\text{Zn}^{2+}$ gave the poorest growth of 37.01±0.6666 mg/50 cm$^3$ closely followed and significantly ($P < 0.01$) different by a complete medium without $\text{Fe}^{2+}$ with a mycelial weight of 43.01±0.6667 mg/50 cm$^3$ and the $\text{Mn}^{2+}$-free medium (50.0±0.3333 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 $\text{Mn}^{2+}$-free medium could be due to improper functioning of phosphate group transfer system during cellular respiration (Prescott et al., 2008). A complete medium without $\text{Co}^{2+}$ 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±1.6607 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.

<table>
<thead>
<tr>
<th>pH</th>
<th>Mycelial dry weight (mg/50 cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>18.0±2.033³</td>
</tr>
<tr>
<td>4</td>
<td>73.0±1.0837⁵</td>
</tr>
<tr>
<td>5</td>
<td>106.0±1.4240⁴ab</td>
</tr>
<tr>
<td>6</td>
<td>98.0±0.8505⁴abc</td>
</tr>
<tr>
<td>7</td>
<td>66.0±1.8037³ab</td>
</tr>
<tr>
<td>8</td>
<td>45.1±0.3712⁴c</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are means of three replicates ± 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.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Mycelial dry weight (mg/50 cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>15.5±0.8821⁴a</td>
</tr>
<tr>
<td>25</td>
<td>80.6±1.1155⁴b</td>
</tr>
<tr>
<td>30</td>
<td>145.5±0.0000⁴a</td>
</tr>
<tr>
<td>35</td>
<td>120.6±0.5812⁴b</td>
</tr>
<tr>
<td>40</td>
<td>34.6±5.0000⁴b</td>
</tr>
<tr>
<td>45</td>
<td>20.8±0.6667⁷ab</td>
</tr>
</tbody>
</table>

Data are means of three replicates ± 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.

<table>
<thead>
<tr>
<th>Mineral elements</th>
<th>Mycelial dry weight (mg/50 cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microelements</td>
<td></td>
</tr>
<tr>
<td>Basal medium only (control 1)</td>
<td>18.3±0.2796⁴ab</td>
</tr>
<tr>
<td>Complete medium minus $\text{Co}^{2+}$</td>
<td>57.3±0.0000⁴b</td>
</tr>
<tr>
<td>Complete medium minus $\text{Cu}^{2+}$</td>
<td>45.00±0.6667⁴c</td>
</tr>
<tr>
<td>Complete medium minus $\text{Fe}^{2+}$</td>
<td>43.01±0.6667⁴ab</td>
</tr>
<tr>
<td>Complete medium minus $\text{Mn}^{2+}$</td>
<td>50.01±0.3333⁴a</td>
</tr>
<tr>
<td>Complete medium minus $\text{Zn}^{2+}$</td>
<td>37.01±0.6666⁴ab</td>
</tr>
<tr>
<td>Complete medium with all the elements (control 2)</td>
<td>57.0±1.6607⁴c</td>
</tr>
<tr>
<td>Macroelements</td>
<td></td>
</tr>
<tr>
<td>Basal medium only (control 1)</td>
<td>15.0±2.8868⁷b</td>
</tr>
<tr>
<td>Complete medium minus $\text{Ca}^{2+}$</td>
<td>15.0±0.7676⁷c</td>
</tr>
<tr>
<td>Complete medium minus $\text{K}^+$</td>
<td>48.6±1.4530⁷c</td>
</tr>
<tr>
<td>Complete medium minus $\text{Mg}^{2+}$</td>
<td>50.7±0.4096⁷b</td>
</tr>
<tr>
<td>Complete medium minus $\text{Na}^+$</td>
<td>33.3±0.6667⁷ab</td>
</tr>
<tr>
<td>Complete medium + all elements</td>
<td>53.30±7.264⁷bc</td>
</tr>
</tbody>
</table>

Data are means of three replicates ± 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±0.7676 mg/50 cm$^3$), followed by a complete medium without sodium and potassium with mycelial weights of 33.3±0.6667 mg/50 cm$^3$ and 48.6±1.4530 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 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 ºC 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.

Acknowledgment

The authors are grateful to the Mrs B. Atobatele of the Department of Biological sciences, Bowen University Iwo, for technical assistance provided during the course of this work.

Correspondence to:
Garuba Emmanuel O.
Department of Biological sciences,
Bowen University, Iwo
Osun State, Nigeria
Telephone +234-803-444-4578
e-mail; oluwa.seungaruba@live.com,
oluwa.seungaruba@yahoo.com

References
7. Duncan, D B. Multiple range and multiple F tests. Biometrics 1955; 11:1–42..