Extracellular $\alpha$-L-arabinofuranosidase from *Aspergillus niger* and *A. oryzae*

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**Abstract:** Interest in the $\alpha$-L-arabinofuranosidases (ABFs) increased recently because of their application in the conversion of various hemicellulosic substrates to fermentable sugars for subsequent production of fuel alcohol and other industrial applications. The production of extracellular ABF and some of its properties by locally isolated strains of *Aspergillus niger* and *A. oryzae* were studied. The best culture conditions for the enzyme production were 1% Sugar beet pulp syrup; pH 5.5 for *Aspergillus niger* and 6.0 for *A. oryzae*; 30$^\circ$C for *Aspergillus niger* and 35$^\circ$C for *A. oryzae*; 7 days of incubation for both, finally the nitrogen source is ammonium Sulphate for both. The enzyme from *Aspergillus niger* was purified 8.34 folds from the culture supernatant by ammonium sulfate treatment, 100 gel filtration on Sephadex G column. While the enzyme from *Aspergillus oryzae* was purified 11.37 100folds from the culture supernatant by ammonium sulfate treatment, gel filtration on Sephadex G column. The purified enzyme of both fungi displayed an optimal activity at 50$^\circ$C and pH 4.0. The enzyme was stable at 30-90$^\circ$C and pH 3.5-5.5.

**Key words:** *Aspergillus niger*, *A. oryzae*, $\alpha$-L- arabinofuranosidase.

**INTRODUCTION**

Alpha-L-arabinofuranosidases (EC 3.2.1.55) are among key enzymes of the hemicellulase system which is tremendously useful in bio-bleaching of paper pulp, bioconversion of lignocellulosic material to fermentative products for subsequent production of fuel alcohol (Saha, 2000) and improvement of animal feed-stock digestibility. Alpha-L-arabinofuranosidases are exo-type enzymes, catalyse the hydrolysis of $\alpha$-1,2-, $\alpha$-1,3- and $\alpha$-1,5-L-arabinofuranosidic bonds in hemicelluloses such as arabinoxylan, L-arabinan and other L-arabinose-containing polysaccharides (Kaji, 1984, Beldman *et al.*, 1997 & Polaina and MacCabe (2007).


Solid substrate fermentation (SSF) has gained importance for the production of microbial enzymes due to economical advantages over conventional submerged fermentation (Pandey *et al.*, 1999 and Holker *et al.*, 2004) and due to the possibility of using cheap and abundant agro-industrial wastes as substrate. It can be of special interest in those processes where the crude fermented product may be used directly as the enzyme source (Holker & Lenz, 2005 and Krishna, 2005).
MATERIALS AND METHODS

Fungi:
Two local wild strains of *Aspergillus niger* Tiegh (1867) and *A. oryzae* (Ahlb-*) E.Cohn (1884) were isolated from a composite soil sample collected from agricultural soil from the botanical garden of Biological Sciences Department, Faculty of Education, Cairo, Egypt.). They were selectively isolated by direct soil plate method (Warcup, 1950) from soil amended with 1% sugar beet pulp and were identified according to Moubasher (1993).

Culture Media and Conditions:
Czapek's is used as a basal medium, where sucrose is replaced by 1% syrup of sugar beet pulp (SBP). The solid (SBP) was obtained from the refinery factory of beet sugar at Kafer El-Sheikh, Egypt. The enzyme production was carried out in 250-ml Erlenmeyer flasks containing 50 ml of the basal medium. Medium was sterilized by autoclaving at 121 ºC for 20 min and cooled to room temperature. The initial pH of the medium was about 5.6. One ml of uniformly prepared spore suspension (10⁷ spores ml⁻¹) from 7 d old cultures was used as inoculum, incubated at 30 ºC for 7 d and the mycelium was separated by filtration. α-L-arabinofuranosidase activity and the protein content in the filtrate were determined. In SSF 5 g of SBP was used as carbon source with 10 ml of basal medium for different times of incubation.

Enzyme Assay:
ABF activity was measured using 2.5 mM p-nitrophenyl-α-L-arabinofuranoside (pNPA) as substrate in 50 mM citrate-phosphate (C-P) buffer pH 4.0. Thirty μl of the enzyme solution was incubated with 200 μl of the substrate at 40ºC for 10 min. The reaction was stopped by addition of equal volume of 0.2 M Na₂CO₃. The liberated p-nitrophenol was measured spectrophotometrically at 410 nm. One unit of the enzyme activity is the amount of enzyme which liberates 1 μmol of nitrophenol per min under the assay conditions.

Protein Determination:
Soluble protein in the culture supernatant was estimated according to the method of Lowry *et al.* (1951) using bovine-serum albumin as a standard. The protein content of the purified enzyme was measured by UV absorbance at 280 nm (Markwell *et al.*, 1978) using bovine serum albumin as a standard.

Purification of α-L-arabinofuranosidase:
Culture supernatants (1000 ml) were concentrated by (NH₄)₂SO₄ precipitation (60%). The pellet was resuspended in 20 ml of 50 mM C-P buffer (pH 4.0), mixed gently then centrifuged (1 min, 4000 rev min⁻¹). The resultant supernatant was subjected to column (26 x 1.8 cm) of Sephadex G₂₀₀. The active fractions were pooled and used for further analyses.

Optimum pH and Stability:
The optimal pH was determined in the range from pH 3.0 to 6.0 (50 mM C-P buffer). Stability of the enzyme was investigated in buffer solutions of various pH values (3.0-6.0). The relative activity was determined after incubation for 24 h at 4 ºC under standard assay conditions.

Temperature Optimum and Stability:
The optimum temperature for activity was determined by measuring the enzyme activity after incubation at various temperatures (30-90ºC) at pH 4.0 using 50 mM C-P buffer. Thermostability of the enzyme was determined by incubating the enzyme in 50 mM C-P buffer at pH 4.0 at various temperatures for 30 min and the relative activity was measured under standard assay conditions.

Effect of Prolonged Storage on ABF Activity:
Storage stabilities of the partially purified ABF was studied at 0, 4, 35 and 55ºC for one month, the residual activity of the enzyme was measured under standard assay conditions.

RESULTS AND DISCUSSION

The production of extracellular α-L-arabinofuranosidase by the two experimental fungi (*Aspergillus niger* & *A. oryzae*) was studied as affected by some of the cultural conditions including the concentration, pH value, temperature, incubation period and different nitrogen sources then we tried solid state culture conditions with different incubation periods to obtain the best culture conditions for its production, followed by purification...
of the resulting enzyme with study of some of its properties. It was found that generally *Aspergillus oryzae* gave higher results than that of *Aspergillus niger*.

It was found that 1% of SBP syrup is the best concentration for the production of α-L-arabinofuranosidase by the two experimental fungi (Fig. 1). The specific activity was 1.986 and 2.099 U/mg protein for *Aspergillus niger* & *A. oryzae* respectively. Other researches obtained different results e.g., El-Gindy and Saad, 2003 obtained 2.78 U/mg protein with the thermophilic fungus *Thermomyces lanuginosus*. Other productions by SC with different fungi gave an even much lower yield (Kaji and Yoshihara, 1971; Uesaka *et al*., 1978). Others showed different amounts of productions of α-L-arabinofuranosidase using different organisms (Gomes *et al*., 2000; Degrassi, *et al*., 2003; Chacon-Martinnez *et al*., 2004; Koseki *et al*., 2006; Khandeparker *et al*., 2008; Raweesri *et al*., 2008; Rosli *et al*., 2009 and Di Santo *et al*., 2009).

![Fig. 1: Effect of different concentrations of SBP syrup on α-L-arabinofuranosidase production by *Aspergillus niger* and *A. oryzae.*](image1)

Lower pH value was favored by the two experimental fungi for the production of α-L-arabinofuranosidase in this study. Fig. (2) shows that the best pH value for *Aspergillus niger* was 6.0 while that of *Aspergillus oryzae* was 5.5 where the specific activities were 2.33 and 2.811 U/mg protein for *Aspergillus niger* & *A. oryzae* respectively. Similar results were obtained by many authors using different microorganisms (Kaji *et al*., 1969; Gilead and Shoham, 1995; Degrassi *et al*., 2003; Abelardo and Clara, 2003 and Rahman *et al*., 2003).

![Fig. 2: Effect of pH value on α-L-arabinofuranosidase production by *Aspergillus niger* and *A. oryzae.*](image2)
Concerning the effect of incubation temperature on the production of α-L-arabinofuranosidase the two experimental fungi differ slightly (Fig. 3) where the best incubation temperature for *Aspergillus niger* was 30°C (3.345 U/mg protein) and that of *A. oryzae* was 35°C (3.591 U/mg protein). This was in consistence with the findings of several authors (Le Clinche et al., 1997; Kaneko et al., 1998; Saha and Bothast, 1998; De Ioannes et al., 2000; Hashimoto and Nakata, 2003; Rahman et al., 2003; Chacon-Martinnez et al, 2004; Koseki et al., 2006 and Fritz et al, 2008).

![Fig. 3: Effect of temperature on α-L-arabinofuranosidase production by *Aspergillus niger* and *A. oryzae.*](image)

The two experimental fungi showed the highest α-L-arabinofuranosidase after 7 days where the specific activities were 2.49 and 3.78 U/mg protein for *Aspergillus niger* & *A. oryzae* respectively (Fig. 4). This may be due to these fungi are mainly mesophilic. Slightly different results were obtained by many authors (Le Clinche et al., 1997; Kaneko et al., 1998; Saha and Bothast, 1998; De Ioannes et al., 2000; Hashimoto and Nakata, 2003; Rahman et al., 2003; Chacon-Martinnez et al, 2004; Koseki et al., 2006 and Fritz et al, 2008).

![Fig. 4: Effect of incubation period on α-L-arabinofuranosidase production by *Aspergillus niger* and *A. oryzae.*](image)

Different nitrogen sources were tried with the two experimental fungi including organic and inorganic examples. The results (Fig. 5) showed that ammonium sulphate \((\text{NH}_4)_2\text{SO}_4\) was the best one, where they produced 2.97 and 4.58 U/mg protein for *Aspergillus niger* & *A. oryzae* respectively. This may attributed to the low pH value preferred by these fungi. Different nitrogen sources including \((\text{NH}_4)_2\text{SO}_4\) also tried by many authors (Roche, et al., 1995; De Ioannes et al., 2000; Chinnathambi, & Lachke, 1995; Lauruengtana, & Pinphanichakarn, 2006 and Martinez. et al., 2006).
We studied also the effect of SSF cultivation on the production of \( \alpha \)-L-arabinofuranosidase by the two experimental fungi. The results (Fig. 6) showed it much more promote the production of \( \alpha \)-L-arabinofuranosidase after short period, thus only four days were enough to produce 3.85 and 4.87 U/mg\(^{-1}\) protein for \textit{Aspergillus niger} & \textit{A. oryzae} respectively. It is well known that solid substrate fermentation (SSF) has gained importance for the production of microbial enzymes due to economical advantages over conventional submerged fermentation (Pandey \textit{et al.}, 1999 and Holker \textit{et al.}, 2004) and due to the possibility of using cheap and abundant agro-industrial wastes as substrate. It can be of special interest in those processes where the crude fermented product may be used directly as the enzyme source (Holker & Lenz, 2005 and Krishna, 2005).

The enzyme of \textit{Aspergillus niger} was purified by precipitation with \((\text{NH}_4)_2\text{SO}_4(60\%)\), with 72.15\% yield and 2.074 purification fold (Table 1), followed by gel filtration onto Sephadex G\textsubscript{200} to give 32.87 U mg\(^{-1}\)protein with 19.22 yield and 8.34 purification fold, while the enzyme of \textit{Aspergillus oryzae} was purified by precipitation with \((\text{NH}_4)_2\text{SO}_4(60\%)\), with 83.0 \% yield and 2.19 purification fold (Table 2), followed by gel filtration onto Sephadex G\textsubscript{200} to give 47.04 U mg\(^{-1}\)protein with 27.17 yield and 11.37 purification fold.

**Fig. 5:** Effect of different nitrogen sources on \( \alpha \)-L-arabinofuranosidase production by \textit{Aspergillus niger} and \textit{A. oryzae}.

**Fig. 6:** Effect of incubation time on the production of \( \alpha \)-L-arabinofuranosidase in SSF medium by \textit{Aspergillus niger} and \textit{A. oryzae}.
Fig. 7: Purification of α-L-arabinofuranosidase from *Aspergillus niger* by gel filtration through Sphadex G-100.

Fig. 8: Purification of α-L-arabinofuranosidase from *Aspergillus oryzae* by gel filtration through Sphadex G-100.

Fig. 9: pH profiles and pH stabilities of α-L-arabinofuranosidase from *Aspergillus niger*. 
Fig. 10: pH profiles and pH stabilities of α-L-arabinofuranosidase from *Aspergillus oryzae*.

Fig. 11: Temperature profiles and pH stabilities of α-L-arabinofuranosidase from *Aspergillus niger*.

Fig. 12: Temperature profiles and pH stabilities of α-L-arabinofuranosidase from *Aspergillus oryzae*. 
Table 1: Purification steps of α-L-arabinofuranosidase produced by Aspergillus niger.

<table>
<thead>
<tr>
<th>Purification steps</th>
<th>Total protein (mg)</th>
<th>Total activity (U)</th>
<th>Specific activity (U mg⁻¹)</th>
<th>Yield (%)</th>
<th>Purification fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude enzyme</td>
<td>249.55</td>
<td>983.69</td>
<td>3.94</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ (60%)</td>
<td>86.63</td>
<td>709.32</td>
<td>8.18</td>
<td>72.15</td>
<td>2.07</td>
</tr>
<tr>
<td>Sephadex G₁₀₀</td>
<td>5.77</td>
<td>189.71</td>
<td>32.87</td>
<td>19.28</td>
<td>8.34</td>
</tr>
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</table>

Table 2: Purification steps of α-L-arabinofuranosidase produced by Aspergillus oryzae.

<table>
<thead>
<tr>
<th>Purification steps</th>
<th>Total protein (mg)</th>
<th>Total activity (U)</th>
<th>Specific activity (U mg⁻¹)</th>
<th>Yield (%)</th>
<th>Purification fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude enzyme</td>
<td>262.8</td>
<td>1088.0</td>
<td>4.14</td>
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<td>1</td>
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<tr>
<td>(NH₄)₂SO₄ (60%)</td>
<td>99.72</td>
<td>903.2</td>
<td>9.057</td>
<td>83.0</td>
<td>2.19</td>
</tr>
<tr>
<td>Sephadex G₁₀₀</td>
<td>6.28</td>
<td>295.7</td>
<td>47.08</td>
<td>27.17</td>
<td>11.37</td>
</tr>
</tbody>
</table>

ABFs have been purified from culture filtrates using (NH₄)₂SO₄ precipitation, gel filtration (Kaji et al., 1981; Higashi, 1983; Kaji, 1984; De Ioannes et al., 2000; El-Gindy and Saad, 2003).

The optimum pH for ABF from Aspergillus niger was 4.0 (Fig. 9). The pH stability exhibited by the enzyme was between 3.5-6.0. While, the optimum pH for ABF from Aspergillus niger was 4.0 (Fig. 10). The pH stability exhibited by the enzyme was between 3.5-6.0. The optimum temperature of the enzyme produced by Aspergillus niger & Aspergillus oryzae was 50 °C and the thermostability exhibited by the enzyme was between 30-80°C (Figs. 11 and 12). Other fungal ABFs have a broad range of pH and temperature dependence, with optimum activity between 3.0 and 6.9 and temperature from 40 to 70°C (Kaji, 1984). ABF from Aspergillus awamori has an optimum activity at pH 5.0 and 50°C (Kormelink et al., 1991); the enzyme from Aspergillus nidulans optimum activity was observed at pH 4.0 and 65°C (Fernandez-Espinar et al., 1994); two ABFs were purified with an optimal activity at pH 4.0 for both and 55°C for one and 60°C for the other (Filho et al., 1996); ABF from Aureobasidium pullulans has a maximal activity at pH 4.0-4.5 and 75°C (Saha and Bothast, 1998). On the contrary, ABF purified from Rhodotorula flavia has an optimal activity at pH 2.0 and 30°C (Uesaka et al., 1978).

The present work demonstrated that high levels of α-L-arabinofuranosidase activity could be produced by the local wild strain of Aspergillus oryzae grown on low cost agricultural by-products and inorganic nitrogen sources. The enzyme was active at considerably high temperature and it had both high thermal and broad range of pH stability. The findings suggested that this enzyme will be suitable for industrial applications in food and feed processing including saccharification of hemicellulosic residues which are renewable resources to fermentable sugars for the production of fuel ethanol as well as other useful fermentation products.

REFERENCES


