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Production, Partial Purification and Some Properties of α-L-Arabinofuranosidase from *Chaetomium thermophile* and *Talaromyces thermophilus*

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THE PRODUCTION of extracellular α-L-arabinofuranosidase (ABFase), partial purification and some of its properties by locally isolated strains of *Chaetomium thermophile* and *Talaromyces thermophilus* were studied. The best static culture conditions for the enzyme production were 2% sugar beet pulp; pH 5.0 and 45ºC for both strains. The best nitrogen source is ammonium sulphate for *C. thermophile* and yeast extract for *T. thermophilus*. The enzyme was partially purified from the culture supernatant by precipitation with ammonium sulfate treatment, gel filtration on Sephadex G_{100}, with purification fold 15.89 and 8.32 for *C. thermophile* and *T. thermophilus*, respectively. The purified enzyme of both fungi displayed an optimal activity at 50 ºC. The enzyme was stable at temperatures between 30-70 ºC. The optimum pH for *C. thermophile* was 5.5 and the enzyme was stable at pH between 3.0 – 6.5. While the optimum pH for *T. thermophilus* was 6.5 and the enzyme was stable at pH between 3.0 – 7.5.

Keywords: *Chaetomium thermophile* , *Talaromyces thermophilus* , α-L- arabinofuranosidase.

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 lignocelluloses 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 α-1,2-, α-1,3- and α-1,5-L-arabinofuranosidic bonds in hemicelluloses such as arabinoxylan, L-arabinan and other L-arabinose-containing polysaccharides (Kaji, 1984; Beldman *et al.*,1997 and Polaina & MacCabe , 2007).

Alpha-L-arabinofuranosidases with their synergistic action with other lignocelluloses degrading enzymes are the promising tools in agro-industrial processes (Saha, 2000). Owing to their industrial importance, a variety of ABFase have been purified from various sources such as bacteria, fungi and plants. Among fungi reported as ABFase producers are *Trichoderma reesei* (Poutanen, 1988 and Roche *et al*., 1995), *Aspergillus niger* (Rombousts *et al*., 1988; Gunata *et al*., 1990

and van der Veen *et al*., 1993), *Aspergillus awamori* (Kormelink *et al*., 1991 and Kaneko *et al*., 1998), *Aspergillus nidulans* (Ramon *et al*., 1993 and Fernandez-Espinar *et al*., 1994), *Aspergillus terreus* (Luonteri *et al*., 1995 and Le Clinche *et al*., 1997), *Penicillium capsulatum* (Filho *et al*., 1996), *Aureobasidium pullulams* (Saha & Bothast,1998), *Penicillium purpurgenum* (De Ioannes *et al*., 2000) *Aspergillus oryzae* (Hashimoto & Nakata, 2003) *Rhizomucor pusillus* (Rahman *et al*., 2003) *Thermomyces lanuginosus* (El-Gindy & Saad, 2003) *Fusarium oxysporum* (Chacon-Martinnez *et al*., 2004), *Aspergillus kawachii* (Koseki *et al*., 2006) and *Penicillium purpurogenum* (Fritz *et al*., 2008). ABFase have received much attention because of their potential application in food (Gunata *et al*., 1990 and Worstald *et al*., 1994); feed (Greve *et al*., 1984 and Bezalel *et al*., 1993); pulp and paper industries (Gomes *et al*., 2000). The aim of this study is the production of ABFase from newly isolated potent local strains of *C. thermophile* and *T. thermophilus.*

Materials and Methods

Fungi

Two local wild strains of *Chaetomium thermophile* Latouche and *Talaromyces thermophilus* , 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 static culture conditions

Czapek's solution is used as a basal medium, where sucrose is replaced by 1% of sugar beet pulp (SBP), obtained from the refinery factory of beet sugar at Kafecelsheikh, Egypt. The enzyme production was carried out statically in 250-ml Erlenmeyer flasks containing 50 ml of the basal medium. Medium was sterilized by autoclaving at 121 °C 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 (10^5 spores ml^{-1}) from 7 days old cultures was used as inoculum, incubated at 30°C for 7 days and the mycelium was separated by filtration. α-L-arabinofuranosidase activity and the protein content in the filtrate were determined. The culture filtrate was used as the crude enzyme preparation.

Enzyme assay

ABFase activity was measured using 2.5 mM ρ-nitrophenyl-α-L-arabinofuranoside (ρNPA) as substrate in 50 mM citrate-phosphate (C-P) buffer pH 4.0. Three hundred 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 Na_2CO_3. The liberated ρ-nitrophenol was measured spectrophotometrically at 410 nm. One unit of the enzyme activity was defined as the amount of enzyme which released 1 µmol of nitrophenol per min under these assay conditions.

Protein determination

Soluble protein in the culture filtrate 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.

Partial purification of the enzyme

Culture supernatants (1000 ml) were concentrated by $(NH_4)_2SO_4$ precipitation (60%) at room temperature. The pellets were 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 G_{100}. The active fractions were pooled and used for further analyses.

Some properties of the purified α-L-arabinofuranosidase

pH optimum and stability.

The effects of pH on the activity and stability were determined in the range from pH 3.0 to 6.0 (50 mM C-P buffer). The enzyme stability was determined after storage incubation for 24 hr at 4 ºC.

Temperature optimum and stability

The optimum temperature for activity was determined by measuring the enzyme activity after incubation at various temperatures (30-90 ºC) 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.

Effects of prolonged storage on ABFase activity

Storage stabilities of the partially purified ABFase was studied at 0, 4, 35 and 55°C for one month, the residual activity of the enzyme was measured under standard assay conditions.

Effects of some chemical agents and metal cations on ABFase activity

The effects of several metals and chemical agents on the partially purified α-L-arabinofuranosidase activity were determined. The partially purified ABFase was incubated for 5 min with 1mM and 5 mM, then the relative activity of ABFase was determined.

Results and Disscusion

The production of extracellular α-L-arabinofuranosidase by the two experimental fungi (*C. thermophile* & *T. thermophilus*) was optimized at different concentrations of SBP, pH value, temperature, incubation period and different nitrogen sources, followed by purification and study of some of its properties.

It was found that 2% of SBP is the best concentration for the production of α-L-arabinofuranosidase by the two experimental fungi (Fig. 1). The specific activity was 1.65 and 1.21 U/mg^{-1} protein for *C. thermophile* & *T. thermophilus*, respectively. El-Gindy & Saad (2003) obtained 2.78 U/mg^{-1} protein with the

thermophilic fungus *Thermomyces lanuginosus*., *i.e* they obtained higher specific activity than ours. Other fungi gave an even much lower yield (Kaji & Yoshihara, 1971 and 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).

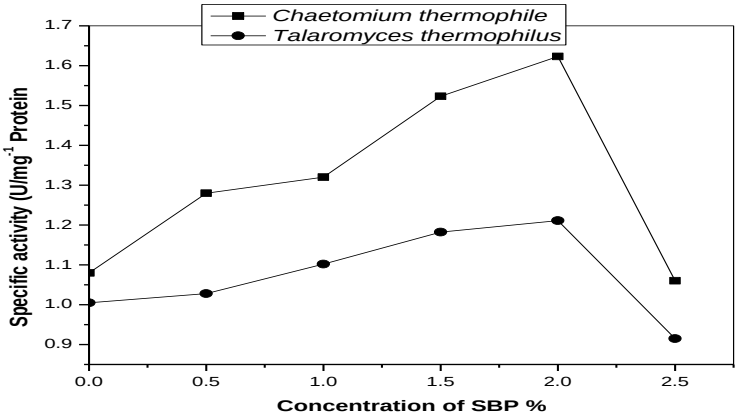

Fig.1. Effect of different concentrations of SBP on ABFase production by *C. thermophile* & *T. thermophilus.*

Lower pH value was favored by the two experimental fungi for the production of α-L- arabinofuranosidase in this study. Figure 2 shows that the best pH value was 5.0 for both strains, on the other hand the specific activities were 2.126 and 1.749 U/mg^{-1} protein for *C. thermophile* & *T. thermophilus*, respectively. Similar results were obtained by many authors using different microorganisms (Gilead & Shoham, 1995; Degrassi *et al*., 2003; Abelardo & Clara, 2003 and Rahman *et al*., 2003).

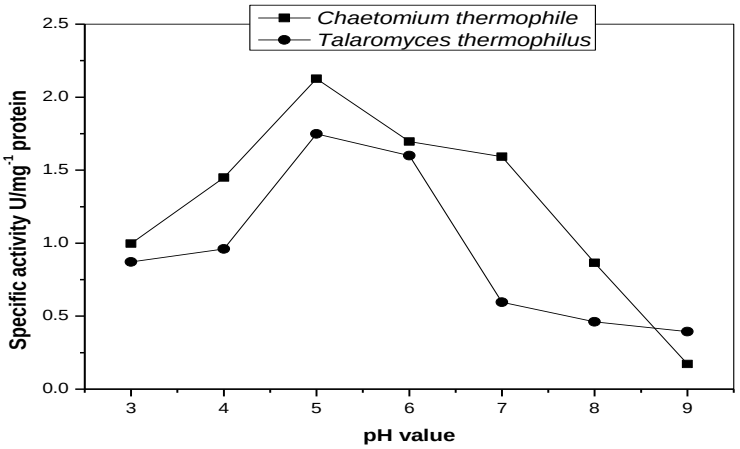

Fig. 2. Effect of pH on the production of ABFase by *C. thermophile* & *T. thermophilus.*

The effect of incubation temperature on the production of α-L-arabinofuranosidase using the two experimental fungi showed high enzyme yield at 45 ºC (Fig. 3). The specific activity for *C. thermophile* was 2.092 U/mg^{-1} protein and that of *T. thermophilus* was 2.85 U/mg^{-1} protein. This was in consistence with the findings of several authors (Le Clinche *et al*., 1997; Kaneko *et al*., 1998; Saha & Bothast,1998; De Ioannes *et al*., 2000; Hashimoto & Nakata, 2003; Rahman *et al*., 2003;Chacon-Martinnez *et al*., 2004; Koseki *et al*., 2006 and Fritz *et al*., 2008) . This may be due to similarity in thermophily of tested organisms.

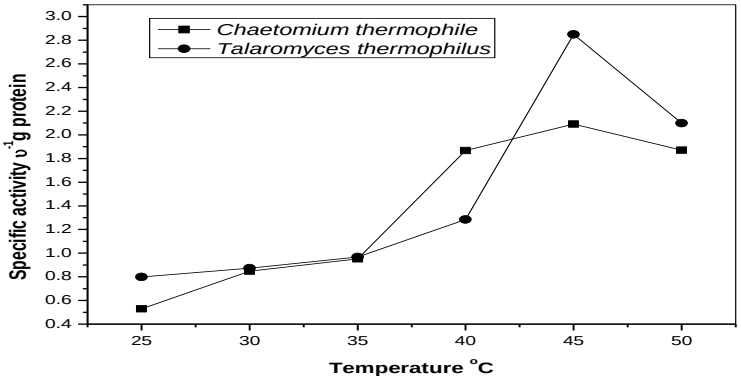

Fig. 3. Effect of temperature on the production of ABFase by *Chaetomium thermophile* & *Talaromyces thermophilus.*

Different nitrogen sources were tried with the two experimental fungi including organic and inorganic examples. The results (Fig.4) showed that ammonium sulphate $(NH_4)_2SO_4$ was the best for *C. thermophile*, producing 2.791 U/mg^{-1} protein and yeast extract was the best for *T. thermophilus* producing 2.821 U/mg^{-1} protein. Different nitrogen sources including $(NH_4)_2SO_4$ also tried by many authors (Roche *et al*., 1995; De Ioannes *et al*., 2000; Chinnathambi & Lachke, 1995; Lauruengtana & Pinphanichakarn, 2006 and Martínez *et al*., 2006).

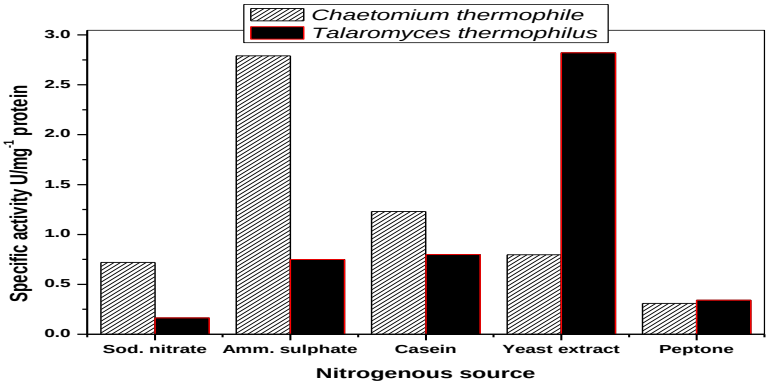

Fig. 4. Effect of different nitrogen sources on the production of ABFase by *C. thermophile* & *T. thermophilus.*

Purification and enzymatic properties of ABFase

ABFase have been purified from culture filtrates using $(NH_4)_2SO_4$ precipitation, gel filtration (Higashi, 1983; Kaji, 1984; De Ioannes *et al*., 2000 and El-Gindy & Saad, 2003). The enzyme of *C. thermophile* was purified by precipitation with $(NH_4)_2SO_4$ (60%), with 55.8% yield and 3.29 purification fold (Table 1), followed by gel filtration onto Sephadex G_{100} to give 25.14 U mg^{-1} protein with 57.42 yield and 15.89 purification fold (Fig. 5), while the enzyme of *T. thermophilus* was purified by precipitation with $(NH_4)_2SO_4$ (60%), with 45.55 % yield and 3.25 purification fold (Table 2), followed by gel filtration onto Sephadex G_{100} to give 17.22 U mg^{-1}protein with 85.13 yield and 8.32 purification fold (Fig. 6).

TABLE 1. Purification steps of ABFase produced by *C. thermophile.*

Purification Steps	**Total protein (mg)**	**Total activity (U)**	**Specific activity (U mg^{-1})**	**Yield (%)**	**Purification fold**
Crude enzyme	595.1	941.6	1.582	100	1
$(NH_4)_2SO_4$ (60%)	101.02	525.85	5.206	55.8	3.29
Sephadex G_{100}	21.509	540.7	25.14	57.42	15.89

TABLE 2. Purification steps of ABFase produced by *T. thermophilus* .

Purification Steps	**Total protein (mg)**	**Total activity (U)**	**Specific activity (U mg^{-1})**	**Yield (%)**	**Purification fold**
Crude enzyme	112.7	233.1	2.068	100	1
$(NH_4)_2SO_4$ (60%)	157.9	1061.8	6.725	45.55	3.25
Sephadex G_{100}	11.52	198.46	17.22	85.13	8.32

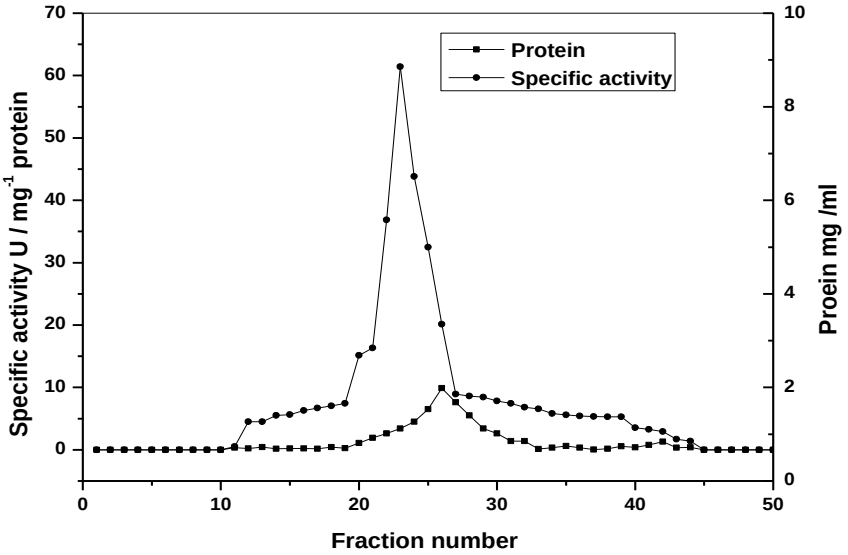

Fig. 5. Purification of ABFase from *C. thermophile* by gel filtration through Sphadex G_{100}.

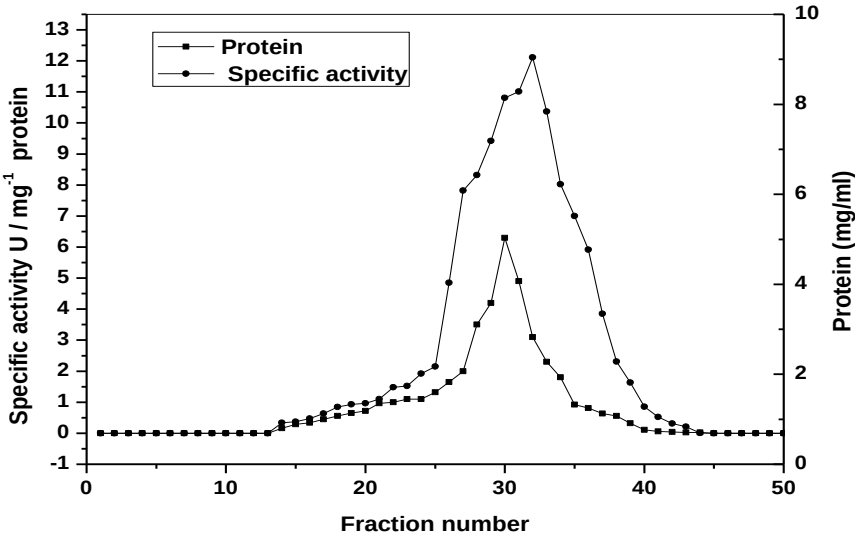

Fig. 6. Purification of ABFase from *T. thermophilus* by gel filtration through Sphadex G_{100}.

The optimum temperature of the enzyme produced by *C. thermophile* & *T. thermophilus* was 50 ºC and the thermostability exhibited by the enzyme was between 30-70 ºC for 30 min (Figs. 7 & 8). The optimum pH for ABFase from *Chaetomium thermophile* was 5.5 (Fig. 9). The pH stability exhibited by the enzyme was between 3.0-6.5. While, the optimum pH for ABFase from *T. thermophilus* was 6.5 (Fig. 10). The pH stability exhibited by the enzyme was between 3.0-7.5. Other fungal ABFase

have a broad range of pH and temperature dependence, with optimum activity between 3.0 and 6.9 and temperature from 40 to 70 ºC (Kaji, 1984). ABFase from *Aspergillus awamori* has an optimum activity at pH 5.0 and 50 ºC (Kormelink *et al.*, 1991); the enzyme from *Aspergillus nidulans* optimum activity was observed at pH 4.0 and 65 ºC (Fernandez-Espinar *et al.*, 1994); two ABFase were purified with an optimal activity at pH 4.0 for both and 55 ºC for one and 60 ºC for the other (Filho *et al.*, 1996); ABFase from *Aureobasidium pullulans* has a maximal activity at pH 4.0-4.5 and 75 ºC (Saha & Bothast, 1998). On the contrary, ABFase purified from *Rhodotorula flava* has an optimal activity at pH 2.0 and 30 ºC (Uesaka *et al.*, 1978).

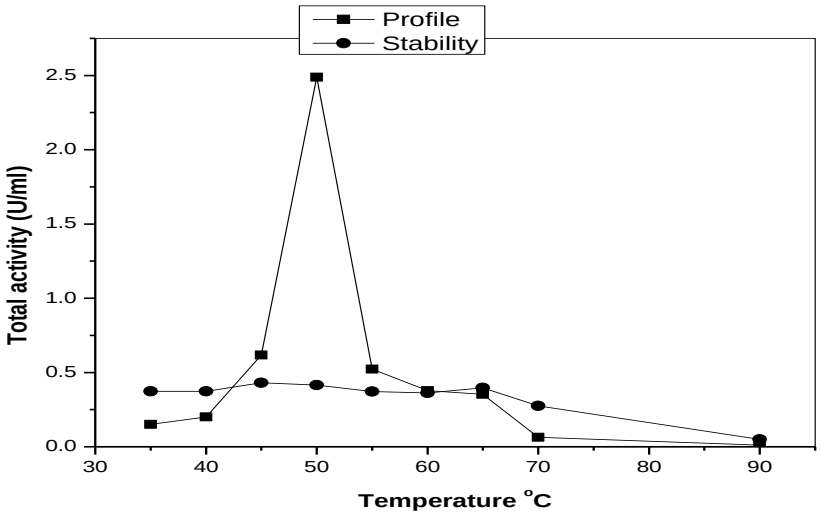

Fig. 7. Temperature profiles and temperature stabilities of ABFase from *C. thermophile.*

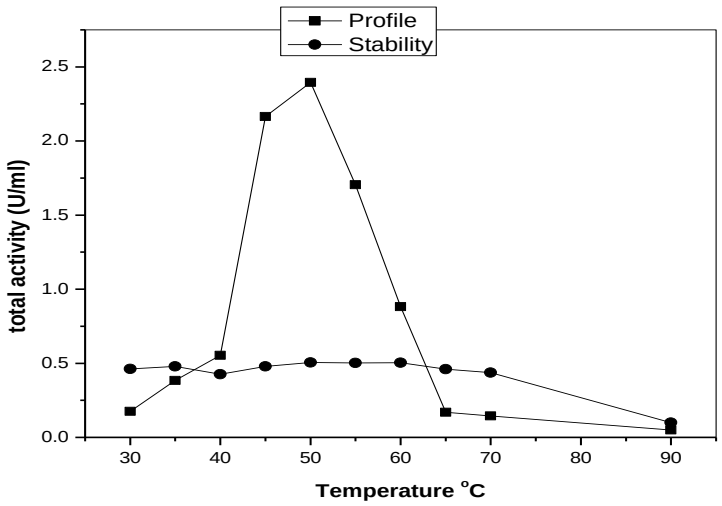

Fig. 8. Temperature profiles and temperature stabilities of ABFase from *T. thermophilus.*

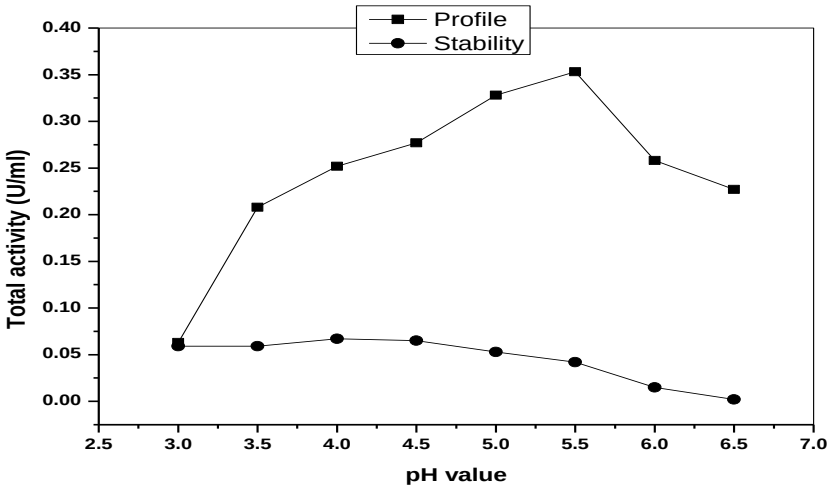

Fig. 9. pH profiles and pH stabilities of ABFase from *C. thermophile.*

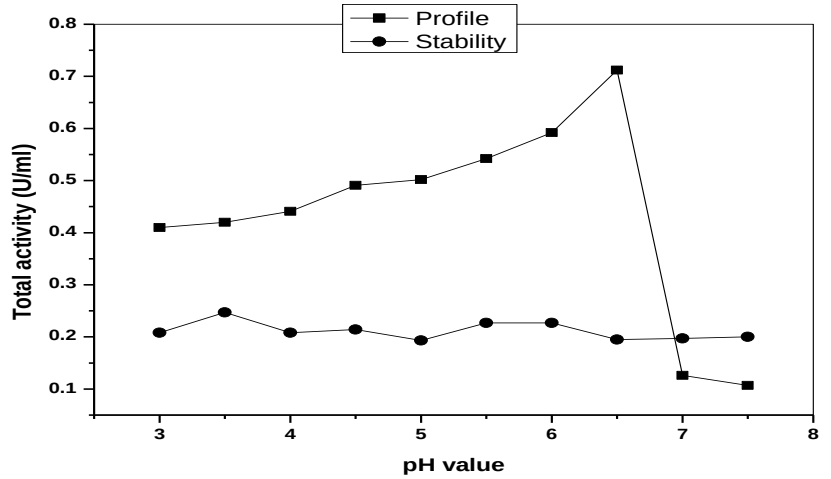

Fig. 10. pH profiles and pH stabilities of ABFase from *T. thermophilus.*

Concerning the effect of enzyme modulators including some metal ions on the activities of the two ABFase (at the concentration of 1and 5 mM), the results (Tables 3 & 4) indicate that divalent ions differ in their amount of effect on ABFase produced by the experimental fungi as follows; ABFase were mostly activated by Zn^{+2}, Ca^{+2} and Co^{+2} where the relative activity reached 301.09%, 261.78% & 226.57%, respectively for *C. thermophile* while in the case of *T. thermophilus* the relative activities for the three cations were 300.27% , 232.71% and 222.90%, respectively. Moderate activation was achieved by Cd^{+2}, Mo^{+2}, Li^{+2} and Mn^{+2} for *C. thermophile* and these cations showed some sort of inhibition for *T. thermophilus*. On the other hand, Hg^{+2} had a mild inhibition for both of the experimental fungi. K^{+} exhibited

inhibition effect on ABFase giving relative activities 21.09% and 14.33% for *C. thermophile* and *T. thermophilus*, respectively while Na^+ gave relative activities of 16.11% and 15.48% for the experimental fungi. The activation effect of some metal ions on ABFase produced by the experimental fungi suggested that these cations are essential for the enzyme action which may be metallo-protein enzymes. Similar results were obtained by Koseki *et al*. (2006), Khandeparker *et al*. (2008), Raweesri *et al* (2008), Rosli *et al*. (2009) and Di Santo *et al*. (2009).

This on the contrary of other molds which not require cofactors for their enzyme action (Gonzalez & Monsan, 1991 and El-Gindy, 2003). Other modulators used in this investigation including sodium arsenate, EDTA, mercaptoethanol, phenyl methyl sulphonyl fluoride and sodium azide severely inhibited ABFase from both of the experimental fungi; where the relative activity did not exceed 20% . This may be attributed to denaturation of the enzyme protein (El-Gindy, 2003).

TABLE 3. Effect of some chemical agents and metal cations on ABFase activity of *Chaetomium thermophile* .

Compound	**Relative activity (%)**	
	Activity at 1 mM	**Activity at 5 mM**
Control	100.0	100.0
Sodium arsenate	0.273	11.41
EDTA	0.195	29.45
Mercaptoethanol	0.378	12.46
Phenyl methyl sulphonyl fluoride	0.292	12.37
Sodium azide	0.221	17.94
Cd^{+2}	3.107	106.23
Mo^{+2}	2.899	105.29
K^+	0.225	21.09
Li^{+2}	0.464	105.02
Zn^{+2}	2.478	301.09
Na^+	0.225	16.11
Ca^{+2}	1.754	261.78
Mg^{+2}	3.818	78.63
Co^{+2}	0.540	226.57
Mn^{+2}	4.557	106.07
Hg^{+2}	0.718	51.78

TABLE 4. Effect of some chemical agents and metal cations on ABFase activity of *Talaromyces thermophilus.*

Compound	Relative activity (%)	
	Activity at 1 mM	Activity at 5 mM
Control	100.0	100.0
Sodium arsenate	0.458	15.76
EDTA	0.254	14.79
Mercaptoethanol	0.351	18.20
Phenyl methyl sulphonyl fluoride	0.397	13.01
Sodium azide	0.279	15.57
Cd^{+2}	0.601	26.63
Mo^{+2}	0.279	27.09
K^{+}	0.412	14.33
Li^{+2}	0.435	69.21
Zn^{+2}	0.452	300.27
Na^{+}	0.349	15.48
Ca^{+2}	3.144	232.71
Mg^{+2}	3.127	53.22
Co^{+2}	2.407	222.90
Mn^{+2}	5.592	92.71
Hg^{+2}	0.718	52.25

Conclusion

The present work demonstrated that high levels of α-L-arabinofuranosidase activity could be produced by the local wild strains of *Chaetomium thermophile* and *Talaromyces thermophilus* grown on low cost agricultural by-products. The enzyme was active at considerably high temperature and it had both high thermal and broad range of pH stability. These 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.

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الإنتاج والتنقية الجزيئية وبعض الخواص لإنزيم ألفا - إلـ أرابينوفيورانوزيديز من فطرتى *كيتوميوم ثيرموفيل* و *تلارومييس ثيرموفيللسس*

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تم دراسة إنزيم ألفا – إلـ أرابينوفيورانوزيديز المنتج خارجيا من فطرتى *كيتوميوم ثيرموفيل* و*تلارومييس ثيرموفيللسس* المعزولتين محليا من التربية المصرية . وقد تم عمل تنقية جزئية للإنزيم ودراسة بعض خواصه وذلك باستخدام المزارع الساكنة .

كانت أفضل الظروف المزرعية لإنتاج الإنزيم هى ٢٪ من لب البنجر كمصدر كربونى والتركيز الأمثل لدرجة أيون الهيدروجين pH هو ٥ و تحت درجة حرارة ٤٥° م لكلا الفطرتين محل الدراسة .

كان أفضل مصدر نيتروجينى لفطرة *كيتوميوم ثيرموفيل* هو كبريتات الأمونيوم بينما كان مستخلص الخميرة هو المصدر النيتروجينى الأمثل لفطرة *تلارومييس ثيرموفيللسس* .

تم تنقية الإنزيم جزئيا بعد ترسيبه باستخدام كبريتات الأمونيوم ثم عمل الترشيح الهلامى على سيفادكس G_{100} وكان معدل التنقية ١٥,٨٩ ضعف لفطرة *كيتوميوم ثيرموفيل* و ٨,٣٢ ضعف لفطرة *تلارومييس ثيرموفيللسس* .

كان النشاط الأمثل للإنزيم المنقى عند درجة حرارة ٥٥° م لكلا الفطرتين كما أنه أظهر ثباتا حراريا من ٣٠° م إلى ٧٠ ° م .

كان التركيز الأمثل لتركيز أيون الهيدروجين للإنزيم المنقى من فطرة *كيتوميوم ثيرموفيل* عند pH ٥,٥ وأظهر ثباتا من ٣- ٦,٥pH .

كان التركيز الأمثل لتركيز أيون الهيدروجين للإنزيم المنقى من فطرة *تلارومييس ثيرموفيللسس* عند pH ٦,٥ وأظهر ثباتا من ٣- ٧,٥ pH .