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Growth Profile and Amylolytic Activity of a Thermophilic Fungus *Aspergillus fumigatus* Isolated from Soil

T.N. Nwagu and B.N. Okolo
Department of Microbiology, University of Nigeria, Nsukka, Nigeria

Corresponding Author: T.N. Nwagu, Department of Microbiology, University of Nigeria, Nsukka, Nigeria

ABSTRACT

Amylases are used in numerous industrial applications and for starch conversion to value added products. The increasing demand for amylase in diverse industrial processes creates the need for isolating microbial strains with novel properties. An amylase producing thermotolerant *Aspergillus fumigatus* was isolated from soil samples after 120 h incubation on potato dextrose agar supplemented with 0.5% starch at 50°C. The ability of the amylase to grow and produce amylase under varying conditions of temperature, starch source, nitrogen source and mineral salts was evaluated. The *Aspergillus* sp. grew well and produced amylase at 55°C though maximum growth and amylase production was recorded after 96 h incubation at 30°C in a mineral medium containing 1% starch and 1.5% organic nitrogen concentration. Sorghum starch as carbon source elicited a better amylase production from the organism. A combination of inorganic (NH_4Cl) and organic (soybean meal) nitrogen sources led to the synthesis of higher concentration of amylase in culture fluid. Amylase was optimally active at pH 6.0 and was acid stable at pH 4.5 retaining over 93% of its activity after 24 h. Optimal temperature for enzyme activity was at 60°C with approximately 65% of enzyme activity retained after 30 min incubation at 70°C. The amylase enzyme was slightly repressed by Fe^{2+} and Mn^{2+} but not inhibited by Cu^{2+}, Co^{2+} and Hg^{2+} at a concentration of 2 mM. The highest hydrolytic activity of the *Aspergillus fumigatus* amylase was recorded for yam, followed by potato and cassava starches.

Key words: Thermotolerant, sorghum, synthesis, soybean, degradation

INTRODUCTION

Starchy foods abound in most countries of the world and are either directlyconsumed or used as raw materials in biotechnological processes. In some of these countries, lack or non-existent proper bioprocess technologies have led to high post harvest losses and major food security challenges (Anthony *et al*., 1996; Omemu *et al*., 2005). Biotechnology apart from enhancing food availability and nutritional quality, will aid long term development of individual countries by terminating the unnecessary loss of valuable raw materials which could be useful in food and other industrial applications (Carlos *et al*., 2002).

Starch is composed of two high molecular weight components including amylose (15-25%) a linear polymer made up of α-1,4-linked glucopyranose residues and amylopectin (75-85%), a branched polymer made up of α-1,4 and α-1,6 linked glucopyranose residues (Dock *et al*., 2008). Amongst starch degrading enzymes are endo-amylases, exo-amylases, debranching enzymes and glycosyltransferases. The α-amylases (EC 3.2.1.1) and glucoamylase (EC 3.2.1.3) are the

endo-amylases and exo-amylases which comprise about 30% of world's enzyme production (Khajeh *et al*., 2006). Depending on the type of amylase, starch is degraded to simple sugars like glucose, maltose or to oligosaccharides, maltooligosaccharides or dextrins (Abou-Elela *et al*., 2009).

Amylases and their products are utilized in a wide range of processes including pharmaceutical, adhesive, sugar, textile, paper, detergent and energy manufacturing processes (Fossi *et al*., 2005). Apart from before mentioned industries, amylases are also employed in fruit juice and brewery industries, in sewage treatment to reduce disposable solid content of sludge and for pretreatment of animal feed to improve digestibility (Kokab *et al*., 2003; Regulapati *et al*., 2007; Saxena *et al*., 2007).

Though enzymes are produced from plants, insects and mammals, microbial enzymes are preferred due to their short growth period, higher productivity and thermostability (Mishra and Behera, 2008). Microbial growth and amylase production is dependent on growth conditions such as type and concentration of carbon and nitrogen substrate, metal ion requirement, pH and temperature of growth (Cherry *et al*., 2004; Ghasemi *et al*., 2010). Though many microorganisms can grow on a wide range of carbon and nitrogen sources, it is economically more viable to utilize cheap and easily available resources as substrates for amylase production (Pandey *et al*., 2000). Brans, straws and flours of different grains and tubers, such as barley, corn, cassava, potato, rice, sorghum and wheat, have been used as carbon sources in the fermentation medium, while protein sources used include soybean meal, yeast extract, peptone, NH_4Cl, $(NH_4)_2SO_4$ and meat extract (Oliveira *et al*., 2007).

Industrial enzymatic hydrolysis is influenced by a number of factors amongst which are environmental conditions of pH, temperature and presence of metal ion (Riaz *et al*., 2007). Given the problems of enzyme inactivation during biotechnology processes there is renewed interest in discovery of thermostable enzymes capable of withstanding high temperatures for a period of time. Several amylase producing *Aspergillus* sp. have been isolated and characterized (Frolova *et al*., 2002; Okolo *et al*., 2001; Prakasham *et al*., 2007). However, the widening of the spectrum of industries which employ amylases and their unique applications necessitate the search for novel amylases.

The aim of this study was to isolate a fungus capable of hydrolyzing a wide range of native starch source on cheap and easily available substrates. A thermotolerant *Aspergillus fumigatus* was isolated from soil collected from Nsukka town in Eastern Nigeria. The ability of the fungus to grow and produce amylase under different environmental conditions was studied. The microorganism was capable of producing large amount of amylase when grown on various native starches including cereal and tuber starches. Effect of pH, temperature and metal ions on the activity and stability of amylase and rate of amylase hydrolysis of different native starches were evaluated.

MATERIALS AND METHODS

Isolation of microorganism: Sample collected in February, 2008 from a honey processing area in Eastern Nigeria was grown in potato dextrose agar supplemented with 0.5% (w/v) starch and incubated at 50°C. Amylolytic isolates were selected by flooding the agar plates with Gram's iodine solution. Isolates which had a higher ratio of clearing zone to colony size were grown in liquid culture and the level of amylase production was determined from cell free culture supernatant fluid. Characterization and identification of the isolate was made following methods outlined for the identification of *Aspergillus* species (Raper and Fennel, 1965).

Nitrogen sources: Soybean meal and cowpea meal was freshly prepared. Both were soaked, soybean meal (overnight), cowpea (1 h) and this was followed by proper washing to remove their test coat. The seeds were promptly dried in an air drier and properly grounded. They were then stored in airtight containers.

Starch substrate: Cassava (*Manihot utillisima*), plantain (*Musa sapientum*), cocoyam (*Xanthosoma saggitifolium*), yam (*Dioscorea rotundata*), corn (*Zea mays*), rice (*Oryzae sativa*) and sorghum (*Sorghum sativa*). Starches were prepared according to standard procedures (Corbishley and Miller, 1984; Watson, 1984). These starches were completely transformed to non-reducing forms by reacting with $NaBH_4$. Other materials used were of analytical grade.

Production of crude enzyme: The inocula were prepared by growing the fungus on Potato Dextrose Agar (PDA). Fermentation medium contained the following in g L^{-1}: $(NH_4)_2SO_4$, 1.4; $KHPO_4$, 2.0; $CaCl_2$, 0.3; $MgSO_4.7H_20$, 0.3; Yeast extract, 10.0; starch, 20.0; Tween-80, 2.0; Mineral solution, 1 mL in deionized water. The mineral solution contained the following in g L^{-1}: $FeSO_4.7H_2O$, 5.0; $MnSO_4$, 1.6; $ZnSO_4.7H_20$, 1.4; $CoCl_2$, 2.0 (Takao *et al*., 1986).

Effect of the cultivation time and temperature on microbial growth and amylase production: To prepare inoculum culture, agar plug of profuse growth of isolate was inoculated into 100 mL Erlenmeyer flask containing 20 mL of the fermentation medium and incubated for 24 h. A suspension containing 10^5 of fungus spores in 1 mL solution served as inoculum. The cultures were incubated at 30, 45 and 55 in a Gallenkamp orbital incubator at 120 rpm for 5 days. The crude enzyme was extracted from growth media by filtration through Whatman No 1 filter paper. The extract was centrifuged at 10,000 rpm (25,900×g) for 15 min at 4°C to remove the suspended particles. Samples were collected daily for the determination of growth rate (OD_{600}), extracellular protein accumulation, reducing sugar production and enzyme activity. Absorbance of reaction solutions was using a Spectronic 20 UV Spectrophotometer.

Enzyme assay: The amylase activity was assayed using a reaction mixture containing 0.5 mL of 1% w/v soluble starch as the substrate in 0.2 M citrate-phosphate buffer at pH 6 and incubated for 10 min at 40°C. Reducing sugar liberated was estimated using the method described by Sandu *et al*. (1987). One unit of amylase (U) was defined as the amount of enzyme that liberated 1 μmole of reducing sugar per minute under the assay conditions.

Effect of organic nitrogen source on amylase production: The effect of 1% of each of the under listed nitrogen sources were studied for their effect on amylase production. These include yeast extract, tryptone, cow blood meal, lablemco powder, casamino acid, soybean meal and cowpea meal.

Effect supplementing organic nitrogen source with inorganic nitrogen: The effects of addition of the 0.2% of the following inorganic nitrogen sources including KNO_3, NH_4Cl, $(NH_4)_2SO_4$ and $NaNO_3$ to medium containing soybean meal (1%) was evaluated. Thereafter, amylase production was assayed as earlier described.

Effect of carbon sources on amylase production: Effect of carbon sources on amylase production was evaluated by substituting 1% soluble starch in the assay mixture with cassava

starch, plantain starch, sorghum starch, sweet potato starch and rice starch. To obtain detailed information about the synthesis of amylolytic enzymes by this organism, the effect of others carbon sources was also checked. These sources include xylose, pectin, mannose, cellulose, glucose, maltose and lactose (1% each).

Effect of metal salt in enzyme production: The effects of the addition of 0.005 M of each of the following salts were studied. These include $FeSO_4.7H_2O$, $CoSO_4$, $ZnSO4.7H_2O$ and $MnSO_4.H_2O$. Result was compared to amylase production when 1 mL of mineral solution containing the following in g L^{-1}: $FeSO_4.7H_2O$, 5.0; $MnSO_4$, 1.6; $ZnSO_4.7H_2O$, 1.4; $CoCl_2$, 2.0 (Takao *et al.*, 1986) was used. A basal medium containing in g L^{-1}: NH_4Cl, 0.4; KH_2PO_4, 2.0; $CaCl_2$, 0.3; $MgSO_4.7H_2O$, 0.3, soybean meal, 10.0; Tween 80, 2.0 and corn starch 20.0 was used.

Characterization of crude enzyme: The effect of temperature on the crude amylase activity and stability was assayed at temperature values ranging from 30-90°C. The reaction mixture containing 0.5 mL of crude enzyme, 0.5 mL of 1% (w/v) soluble starch solution in 5 mL of 0.2 M phosphate buffer pH 6, was incubated for 10 min at the test temperatures. Thereafter, the reaction mixture was promptly cooled on ice before the enzyme activity was assayed as earlier described. To determine enzyme thermostability, the crude enzyme was pre-incubated at various temperatures from 30 to 70°C for 60 min and then promptly chilled on ice, after which the residual activity was determined under normal condition earlier described. The effect of pH on the crude amylase over the range pH 2.5 to 9.0 was also studied. The buffers were prepared as described by McIlvaine for citric acid-Na_2HPO_4 buffer solution, ranging from pH 2.5-7.5 and phosphate buffer, ranging from pH 8.0-9.0. The pH activity profile was determined by incubating 0.5 mL of the enzyme solution with 0.5 mL of 1% (w/v) corn starch in buffer at 40°C for 10 min. Reducing sugar production was assayed as earlier described. The pH stability profile was determined by suspending diluted crude enzyme in appropriate buffer of pH 2.5-9.0 and pre-incubated for 24 h at room temperature. After incubation, residual enzyme activity was assayed as earlier described.

Effect of metal ions on enzyme activity: The effect of various divalent cations (Ca^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Mg^{2+}, Zn^{2+}, Hg^{2+}, Bi^{2+}, Cu^{2+}) on the crude amylase was evaluated. The amylase enzyme was pre-incubated with 2 mM of each metal ion in citrate-phosphate buffer pH 6 at 40°C for 30 min and then assayed for residual amylase activity.

Enzyme Hydrolysis of various starches: The ability of the crude amylase to hydrolyze different native starches was studied using cassava, corn, sorghum, yam and potato starches. Commercially available soluble starch was used as standard. The assay mixture consisted of 10 g L^{-1} of various starch in 0.2 M citrate-phosphate buffer pH 6 and 0.5 mL of the enzyme. Enzyme assay was carried out after incubation at 40°C for 30 min.

Tests were conducted in replicates, standard deviation calculated using Microsoft excel and mean values used for the figures.

RESULTS AND DISCUSSION

Time course of growth and enzyme production of *Aspergillus fumigatus* at different temperatures: *Aspergillus fumigatus* was isolated from soil samples incubated at 50°C. *A. fumigatus* isolate grew best at 30°C; growth progressed rapidly within the first 24 h after which the fungal growth gradually approached the stationary phase as shown in Fig. 1. It was observed

that growth was slightly less at 45°C than 55°C with microbial growth declining after 120 h at 55°C; however growth at 45°C remained at the stationary phase. Figure 2 shows that amylase production was highest at 30°C, followed by 45 and 55°C. While highest amylase production at 30 and 55°C occurred after 96 h, highest production at 45°C was observed after 120 h. In all cases, amylase production by *A. fumigatus* was optimal during the stationary phase of growth.

Effect of organic nitrogen source, carbon source and mineral salts on amylase production of *A. fumigatus*: Different nitrogen sources including soybean meal, casamino acid, yeast extract, tryptone, cowblood meal, lablemco powder and cowpea (1% each) was used as organic nitrogen source for amylase production. Soybean meal elicited the highest production of amylase (1109 U mL^{-1}) and yeast extract (1012 U mL^{-1}) as shown in Table 1. Cowpea meal and tryptone did not favour amylase production and gave the lowest yields of 185 and 546 U mL^{-1}, respectively. Supplementing soybean meal with 0.2% concentration of various inorganic nitrogen salt (KNO_3, NH_4Cl, $(NH_4)_2SO_4$ and $NaNO_3$ had varying effects on amylase production by the fungus. While KNO_3 (1202 U mL^{-1}) and NH_4Cl (1335 U mL^{-1}) stimulated amylase production (Table 2), $NaNO_3$ had an inhibitory effect with observed reduction in extracellular amylase production in the culture fluid from a concentration 1109 U mL^{-1} (only soybean meal as nitrogen source) to 979 U mL^{-1}.

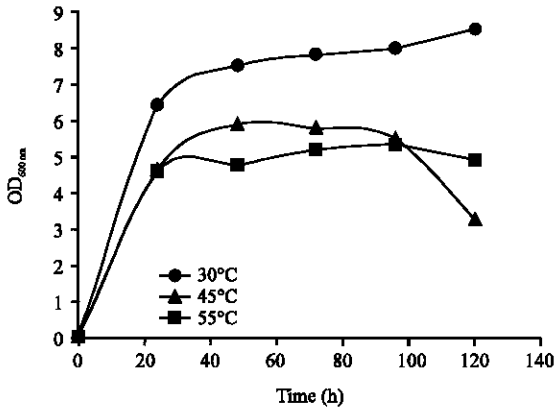

Fig. 1: Effect of time on the growth of *Aspergillus fumigatus* at different temperatures

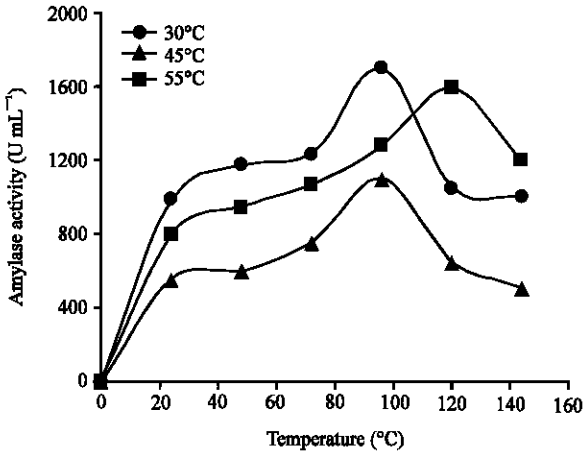

Fig. 2: Effect of temperature on the amylase production of *Aspergillus fumigatus*

Table 1: Effect of different sources of organic nitrogen on amylase production

Nitrogen source (1%)	Amylase activity (U mL^{-1})
Soybean meal	1109±3
Casamino acid	854±3
Yeast extract	1012±8
Tryptone	546±4
Cowblood meal	1001±2
Lablemco powder	638±1
Cowpea meal	185±2

Values are as Mean±SD

Table 2: Effect of supplementation of soybean meal* with different sources of inorganic nitrogen

Nitrogen source (0.2%)	Amylase activity (U mL^{-1})
Potassium nitrate	1202±5
Ammonium chloride	1335±4
Ammonium sulphate	1101±1
Sodium nitrate	979±1

*Soy bean meal (1%) was used as the organic nitrogen source in all cases. Values are as Mean±SD

Table 3: Effect of different carbon sources on amylase production of the *Aspergillus fumigatus*

Carbon sources (1%)	Amylase activity (U mL^{-1})
Cassava starch	1390±2
Plantain starch	1364±1
Corn starch	1596±2
Sorghum starch	1620±4
Potato starch	1132±4
Yam starch	1544±3
Rice starch	1119±1
Soluble starch	1107±2
Cellulose	875±3
Xylose	643±5
Pectin	598±4
Glucose	1016±3
Maltose	1055±2
Mannose	656±2
Lactose	601±4

Values are as Mean±SD

A wide range of starches and sugars were evaluated as to their effects on amylase production by *A. fumigatus*. Sorghum starch gave an amylase yield of 1620 U mL^{-1}, followed by corn starch, 1596 U mL^{-1}; yam starch, 1544 U mL^{-1} and cassava starch, 1390 U mL^{-1} (Table 3). *A. fumigatus* produced amylase while growing on mannose (656 U mL^{-1}), pectin (598 U mL^{-1}), xylose (643 U mL^{-1}) and lactose (601 U mL^{-1}). However, these figures were low compared to amylase production using native starches as carbon source.

Amylase production by *A. fumigatus* increased with increase in sorghum starch to a concentration of 1% after which extracellular production of amylase gradually declined (Result not shown). There was a rapid rise in amylase synthesis in the culture medium of the fungus following an increase in the concentration of soybean meal from 0.5-1.5%, after which there was a rapid decline in enzyme synthesis (Result not shown).

Though $MnSO_4$ (1315 U mL^{-1}), $ZnSO_4$ (1225 U mL^{-1}) and $CoSO_4$ (1333 U mL^{-1}) promoted amylase enzyme production use of a mineral solution containing $FeSO_4$, $MnSO_4$, $CoSO_4$ and $ZnSO_4$ had a more positive effect (Fig. 3). Amylase yield increased from 1080 U mL^{-1} in absence of metal salt to 1689 U mL^{-1} following addition of the mineral salt solution.

Influence of pH, temperature and metal salt on enzyme activity and stability: Optimum pH for amylase produced by *A. fumigatus* was recorded at pH 6.0 with 1% starch as substrate (Fig. 4). The amylase was remarkably acid stable and retained approximately 94% of its activity after 24 h incubation at pH 4.5 to 6.5 and 64% activity at pH 4.

Figure 5 shows that there was a gradual rise in amylase activity with increase in temperature, till an optimum of 60°C was reached, followed by a rapid decline in amylase activity. The crude amylase extract retained over 65% of its activity at 70°C after 30 min incubation.

Catalytic function of the crude amylase was stimulated by Ca^{2+} (133%), Cu^{2+} (123%) and to a lesser extent Zn^{2+} (115%), Bi^{2+} (114) at 2 mM concentrations as illustrated in Table 4. Mn^{2+} (88%) and Fe^{2+} (97%) slightly inhibited the amylase activity.

Relative rate of hydrolysis of different starches by the amylase: The rate of the amylase hydrolysis of different native starches was evaluated. Soluble starch was used as the control with

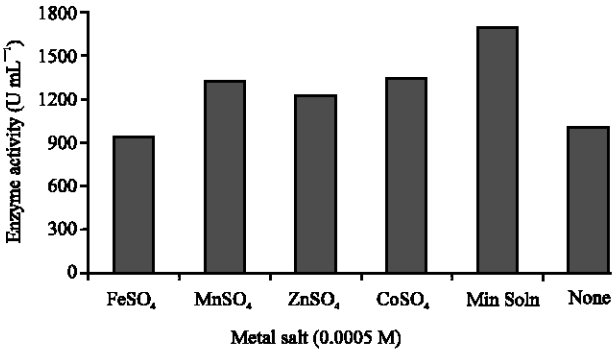

Fig. 3: Effect of utilization of various mineral salts on amylase production by *A. fumigatus*. Concentration of metal salt (0.005 M) each, mineral solution contained all four in 1 mL solution Min Soln = Mineral solution

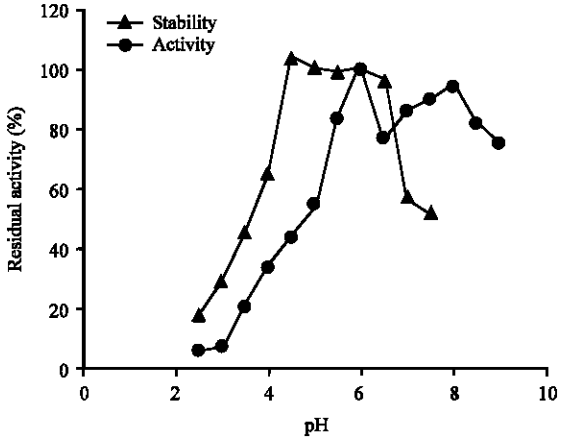

Fig. 4: Effect of amylase activity and stability of *A. fumigatus* on pH. Residual activity at pH 6 was regarded as 100%. (Symbols: circle stands for activity; triangle stands for stability)

the value was taken as 100%. Figure 6 showed that the amylase from *Aspergillus fumigatus* efficiently hydrolyzed yam starch (159%), potato starch (128%) and cassava starch (115%) and corn starch (104%).

Aspergillus fumigatus isolated from soil sample grew well and produced amylase at 30, 45 and 55°C. The ability to grow and metabolize at 55°C indicate that the organism is a facultative thermophile. This is interesting as few fungi are able to survive prolonged exposure to temperatures above 45°C (Leveque *et al*., 2000). Maximum enzyme yield was achieved at 30°C after 96 h which did not correspond with the work of Cherry *et al*. (2004). Type of nitrogen source greatly influenced amylase production by the fungus which corresponded with previous works of Frolova *et al*. (2002) that use of yeast extract in place of peptone for amylase production from *A. flavipes* resulted in a shift from synthesis of amylolytic complexes I and II to only amylolytic complex II. *A. niger* produced a higher amylase when cultivated in the presence of peptone, casamino acid or yeast extract as nitrogen source (Hernandez *et al*., 2006). Present results showed that synthesis of amylase was highest when soybean meal was used as nitrogen source, showing that soybean meal was a better nitrogen source than yeast extract for amylase production by *A. fumigatus*. Further

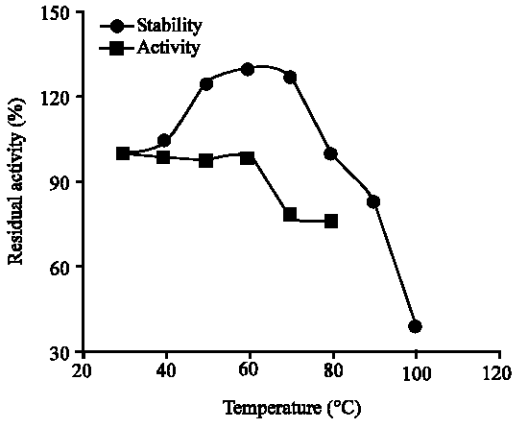

Fig. 5: Dependence of the activity and stability of *A. fumigatus* on temperature (Symbols: circles stands for amylase activity; square for amylase stability). Activity at 30°C was taken as 100%

Table 4: Effect of different cations on the activity of the crude amylase

Metal salt concentration (2 mM)	Relative activity (%)
$BiSO_4$	114±4
$MgSO_4.6H_2O$	107±6
$ZnSO_4.7H_2O$	115±1
$MnSO_4.H_2O$	88±3
$HgCl_2$	110±5
$CuSO_4$	123±2
$CaCl.2H_2O$	133±4
$CoCl.6H_2O$	101±1
$FeSO_4.7H_2O$	97±3
None	100±5

Values are as Mean±SD

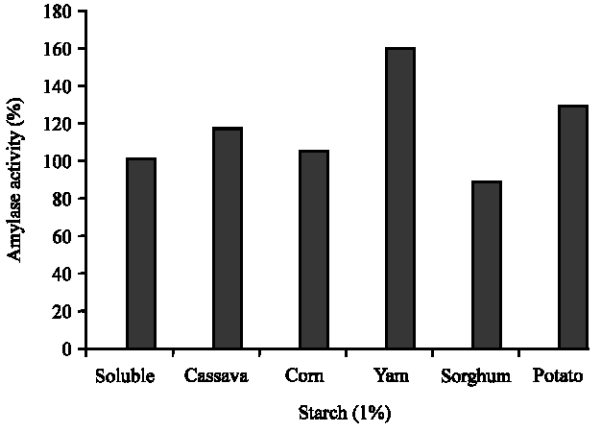

Fig. 6: Relative rate of hydrolysis of different native starches by amylase of *A. fumigatus*. Amylase was incubated with various native starches for 30 min at 40°C. Value for soluble starch was taken as 100% hydrolysis

work was therefore carried out using soybean meal. Supplementing soybean with NH_4Cl led to the synthesis of a higher extracellular amylase in the culture fluid of the organism. Present findings are in full agreement with the report by Aiyer (2004) that combining both inorganic and organic nitrogen source gave increased amylase productivity. Starch source and variation in molecular structure of starch greatly influence amylase induction in microorganisms (Gomes *et al*., 2005). While non-metabolizable sugars such as maltose, xylose and lactose slightly favoured enzyme production, cheap undefined native sources of starch elicited a higher rate of amylase production from *A. fumigatus*. This is in agreement with Nguyen *et al*. (2000) and Peixoto *et al*. (2003), starch and its hydrolytic products were reported to be the induce amylase compared to other carbon sources. Native undefined sources of starch are cheaper and easily accessible; therefore the ability to use them as carbon sources is an advantage over purified starch forms. Metal ions are incorporated into amylase during enzyme synthesis and these ions contribute to the structural and catalytic functions of the enzyme. Rate of amylase production by the fungus was dependent on type and concentration of metal ions present in the growth medium which is in agreement with the report of Poli *et al*. (2009). However our report did not correspond with the work. Ezeji *et al*. (2005). where Co^{2+} and Zn^{2+} inhibited amylase production by *Geobacillus thermodenitrificans* HRO10. Use of mineral salt solution was better and induced the synthesis of higher amylase concentration. Present results agree with that of Carvalho *et al*. (2008) that a proper tailoring of culture components and condition is necessary to ensure optimal yield of protein with desirable characteristics.

The amylase exhibited a broad pH stability range, from pH 4 to pH 9 with only small variations in absolute terms. The acid stability of the amylase at pH 4 is also remarkable as most fungi amylases are unstable at low pH (Okolo *et al*., 2001). The amylase showed a high catalytic activity at 70°C though optimal activity was 60°C, showing that the enzyme is thermoactive. This did not correspond with the report of Cherry *et al*. (2004) on *A. fumigatus* glucoamylase with optimum temperature and pH of 35°C and 7, respectively. However, similar results were obtained for α-amylase produced from another thermophilic fungus *Scytalidium thermophilum* (Aquino *et al*., 2003), optimum pH for *Scytalidium thermophilum* was pH 6 and optimum temperature was 60°C, same for *A. fumigatus*. Industrial applications of amylases require high reaction temperatures to

avoid steps such as cooling down starch slurry after gelatinization, to minimize contamination and for optimal process efficiency. With the exception of Mn^{2+} and Fe^{2+}, *A. fumigatus* amylase was not inhibited by most of the metal ions tested at 2 mM concentration. Though Hg^{2+} has been severally reported to inhibit amylase activity (Okolo *et al.*, 2001; Yang and Liu, 2004; Zhang and Zeng, 2008), Hg^{2+} at a concentration of 2 mM did not inhibit amylase activity of amylase from *A. fumigatus*. Similar results were obtained by Oliveira *et al.* (2010). Present results indicate that the amylase of *A. fumigatus* is stable in the presence of trace amounts of heavy metals. This could be an added advantage, as the sensitivity of amylase to heavy metal ions poses problems in some industrial processes due to the metal composition of reactors or presence of metal contaminants in processing medium.

It is obvious that the amylase showed a more efficient catalysis of tuber starches than cereal starches. This is a remarkable feature as large quantities of tuber starches are lost after every harvesting season in the tropics due to lack of proper preservation technologies (Omemu *et al.*, 2005). Sugars and limit dextrins derived from these starches could serve as raw materials in numerous industrial processes. This will subsequently lead to a reduction in the wastage of invaluable raw materials needed in biotechnological processes and serve as an important economic diversion in the developing countries.

CONCLUSION

Considering the easily affordable substrates needed for copious amylase production, its low pH stability, resistance to inactivation by heavy metal ions, high rate of hydrolysis of native starches, the amylase possess properties which could be invaluable for use in biotechnological applications.

REFERENCES

Abou-Elela, G.M., A.N. El-Sersy and S.H. Wefky, 2009. Statistical optimization of cold adapted α-amylase production by free and immobilized cells of *Nocardiopsis aegyptia*. J. Applied Sci. Res., 5: 286-292.

Aiyer, P.V.D., 2004. Effect of C.N ratio on alpha amylase production by *Bacillus licheniformis* SPT 27. Afr. J. Biotechnol., 3: 519-522.

Anthony, O.E., C. Yusuf and M. Murray, 1996. Culture of *Saccharomyces cerevisae* on hydrolysed waste cassava starch for production of baking quality yeast. Enzyme Microb Technol., 18: 519-525.

Aquino, A.C., J.A. Jorge, H.F. Terenzi and M.L. Polizeli, 2003. Studies on a thermostable α-amylase from the thermophilic fungus *Scytalidium thermophilum*. Appl. Microbial. Biotechnol., 61: 323-328.

Carlos, A., L.M. Meire Lelis and B.L. Angélica, 2002. Production and properties of a-amylase from thermophilic Bacillus species. Br. J. Microbiol., 33: 57-61.

Carvalho, R.V., T.L. Côrrea, J.C. Silva, L.R. Mansur and M.L. Martins, 2008. Properties of an amylase from thermophilic *Bacillus* sp. Bazil. J. Microbiol., 39: 102-107.

Cherry, H.M., M.D. Towhid-Hossain and M.N. Anwar, 2004. Extracellular glucoamylase from the isolate *Aspergillus fumigatus*. Pak. J. Biol. Sci., 7: 1988-1992.

Corbishley, D.A. and W. Miller, 1984. Tapioca, Arrow Root and Sago Starches: Production. In: Starch Chemistry and Technology, Whistler, R.L., J.W. Be-Miller and E.F. Paschal (Eds.). Academic Press, New York, USA., ISBN: 9780127462707, pp: 469- 476.

Dock, C., H. Matthias and G. Antranikian, 2008. A thermoactive glucoamylase with biotechnological relevance from the thermoacidophilic euryarchaeon *Thermoplasma acidophilum*. Applied Microbiol. Biotechnol., 78: 105-114.

Ezeji, T.C., A. Wolf and H. Bahl, 2005. Isolation, characterization and identification of *Geobacillus thermodenitrificans* HRO10, an α-amylase and α-glucosidase producing thermophile. Can. J. Microbiol., 51: 685-693.

Fossi, B., F. Tavea and R. Ndjouenkeu, 2005. Production and partial characterization of a thermostable amylase from ascomycetes yeast strain isolated from starchy soils. Afr. J. Biotechnol., 4: 14-18.

Frolova, G.M., A.S Silchenko, M.V. Pivkin and V.V. Mikhailov, 2002. Amylases of the fungus *Aspergillus flavipes* associated with *Fucus evanescens*. Appl. Biochem. Microbiol., 38: 134-138.

Ghasemi, Y., S. Rasoul-Amini, A. Ebrahiminezhad, G. Zarrini and A. Kazemi *et al*., 2010. Halotolerant amylase production by a novel bacterial strain, *Rheinheimera aquimaris*. Res. J. Microbiol., 5: 144-149.

Gomes, E., S.R. Souza, R.P. Grandi and E.D. Silva, 2005. Production of thermostable glucoamylase by newly isolated *flavus* A. 1.1 and *Thermomyces lanuginosus* A 13.37. Braz. J. Microbiol., 36: 75-82.

Hernandez, M.S., M.R. Rodriguez, N.P. Guerra and R.P. Roses, 2006. Amylase production by *Aspergillus niger* in submerged cultivation on two wastes from food industries. J. Food Eng., 73: 93-100.

Khajeh, K., M.M. Shokri, S.M. Asghan, F. Moradian and A. Ghasemi *et al*., 2006. Acidic proteolytic digestion of α-amylase from *Bacillus licheniformis* and *Bacillus amyloliquefaciens*: Stability and flexibility analysis. Enzyme Microbial. Technol., 38: 422-428.

Kokab, S., M. Asghar, K. Rehman, M. Asad and O. Adedyo, 2003. Bio-Processing of banana peel for α-amylase production by *Bacillus subtilis*. Int. J. Agri. Biol., 1: 36-39.

Leveque, E., S. Janecek, B. Haye and A. Belarbi, 2000. Thermophilic archaeal amylolytic enzymes-catalytic mechanism, substrate specificity and stability. Enzyme Microbial Technol., 26: 3-14.

Mishra, S. and N. Behera, 2008. Amylase activity of a starch degrading bacteria isolated from soil receiving kitchen wastes. Afr. J. Biotechnol., 7: 3326-3331.

Nguyen, Q.D., J.M. Rezessy-Szabo and A. Hoschke, 2000. Optimisation of composition of media for the production of amylolytic enzymes by *Thermomyces lanuginosus* ATCC 34626. Food Technol. Biotechnol., 38: 229-234.

Okolo, B.N., S. Ire, L.I. Ezeogu, C.U. Anyanwu and F.J.C Odibo, 2001. Purification and some properties of a novel raw starch-digesting amylase from *Aspergillus carbonarius*. J. Sci. Food Agric., 81: 329-336.

Oliveira, A., L. Oliveira, J. Andrade and A. Junior, 2007. Rhizobial amylase production using various starchy substances as carbon substrates. Braz. J. Microbiol., 38: 208-216.

Oliveira A.N., L.A. Oliveira and J.S. Andrade, 2010. Partial characterization of amylases of two indigenous Central *Amazonian rhizobia* strains. Braz. Arch. Biol. Technol., 53: 35-45.

Omemu, A.M., I. Akpan, M.O. Bankole and O.D. Teniola, 2005. Hydrolysis of raw tuber starches by amylase of *Aspergillus niger* AM07 isolated from the soil. Afr. J. Biotechnol., 4: 19-25.

Pandey, A., P. Nigam, C.R. Soccol, V.T. Soccol and V.T. Singh *et al*., 2000. Advances in microbial amylases. Biotechnol. Applied Biochem., 31: 135-152.

Peixoto, S.C., J.A. Jorge, H.F. Terenzi and T.M. Polizeli, 2003. Rhizopus microsporus var. rhizopodiformis: A thermotolerant fungus with potential for production of thermostable amylases. Int. Microbiol., 6: 269-273.

Poli, A., S. Salerno, G. Laezza, P. Donato, S. Dumontet and B. Nicolaus, 2009. Heavy metal resistance of some thermophilies: Potential use of α-amylase from *Anozybacillus amylolyticus* as a microbial enzymatic bioassay. Res. Microbiol., 160: 99-106.

Prakasham, R.S., C.S. Rao, R.S. Rao and P.N. Sarma, 2007. Enhancement of acid amylase production by an isolated *Aspergillus awamori*. J. Applied Microbiol., 102: 204-211.

Raper, K.B. and D.J. Fennell, 1965. The Genus Aspergillus. Williams and Wilkins, Baltimore, USA., pp: 686.

Regulapati, R., N. Prem Malav and N. Sathyanarayana Gummadi, 2007. Production of Thermostable α-amylases by solid state fermentation-A review. Am. J. Food Technol., 2: 1-11.

Riaz, M., R. Perveen, M.R. Javed, H. Nadeem and M.H. Rashid, 2007. Kinetic and thermodynamic properties of novel glucoamylase from *Humicola* sp. Enzyme Microbial. Technol., 41: 558-564.

Sandu, D.K., K.S. Vilkhu and S.K. Soni, 1987. Production of α-amylase by *Saccharomyces fibuligera*. J. Ferm. Technol., 65: 387-394.

Saxena, K.R., K. Dutt, L. Agarwal and P. Nayyar, 2007. A highly and thermostable alkaline amylase from a *Bacillus* sp. PN5. Biores. Technol., 98: 260-265.

Takao, S., H. Sasaki, K. Kurosawa, M. Tanida and Y. Kamagata, 1986. Production of a raw starch saccharifying enzyme by *Corticium rolfsii*. Agric. Biol. Chem., 50: 1979-1987.

Watson, S.A., 1984. Corn and Sorghum Starches. In: Starch Chemistry and Technology, Whistler, R.L., J.N. Bemille and E.F. Paschall (Eds.). Academic Press, New York, USA., ISBN-10: 0127462708, pp: 417–468.

Yang, C. and W. Liu, 2004. Purification and properties of a maltotriose-producing α-amylase from *Thermobifida fusca*. Enzyme Microbial. Technol., 35: 254-260.

Zhang, J. and R. Zeng, 2008. Purification and characterization of a cold-adapted α-amylase produced by *Nocardiopsis* sp. 7326 isolated from prydz bay, antarctic. Mar. Biotechnol., 10: 75-82.