JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2012. Vol. **6**(3), p. 1327-1332

Impact of Metal Ions on the Degradation of Agrowaste by *Aspergillus terreus*

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(Received: 14 February 2012; accepted: 25 March 2012)

A total of 102 strains of fungi isolated from soil samples in and around Patna. Three of these strains (AB50, AB55, AB109) showing amylase activity were screened for further investigation. Based on morph-physiological characteristics the strain AB55 showing hyper-amylase activity was identified as *Aspergillus terreus.* The extra cellular amylase production could be detected just after 24 hours under shaking condition and a complete hydrolysis of starch in the medium was observed after 96 hours incubation at 28±2^{0}C. Partially purified amylase enzyme showed a maximum activity of 589U/100ml pH 7.0 and at 50^{0}C temperature. This strain showed good growth in presence of Fe^{+2},Ca^{+2},Mg^{+2},Co^{+2} up to a concentration of 5mM. Almost a two fold enhanced activity was observed in the presence of Fe^{+2},$Mg^{+2}$and Co^{+2}. However, the ions like Hg^{+2},Mn^{+2} and Zn^{+2} remarkably decreased the activity of amylase enzymes.

Key words: Amylase activity; *Aspergillus terreus*; Metal ions.

Starch, the primary storage polysaccharide of plants, is degraded by amylolytic enzymes produced naturally by numerous micro organisms (Lin *et al.,* 1997; Kiran *et al*., 2005). Amylase from plants, animals and micro organisms has been studied in great details ever since enzymes were first discovered (Boyer *et al.,* 1972). Amylases are considered to be of great significance in present day in industrial biotechnology. Enzymes from microbial sources have generally met with the increasing industrial demand due to the ease with which they may be extracted and purified. The spectrum of amylase application has widened in many other fields, such as clinical, medical and analytical chemistry, as well as in starch saccharification, textile industries and food brewing and distilleries (Pandey *et al.,* 2000).

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Contamination of sediments and natural aquatic receptors with heavy metals is a major environmental problem all over the world. (Baldrian and Gabriel 2002Gavrilesca, 2004; Malik, 2004; Srivastava and Thakur 2006). The introduction of heavy metal compounds into the environment generally includes morphological and physiological changes in the microbial communities (Vadkertiova and Slavikova, 2006), hence exerting a selective pressure on the micro biota (Verma et.al, 2001). Generally the contaminates sites are the sources of metal resistant micro organisms (Gadd, 1993). In naturally polluted environments, the microbes response to heavy metals toxicity depends on the concentration and on the action of factors such as the type of metal, the nature of the medium and microbial species (Hassen *et al.*, 1998). Fungi and yeast biomasses are known to tolerate heavy metals (Gavrilesca, 2004; Baldrian, 2003).They are the versatile group, as they can adapts and grow under various extreme conditions of pH, temperature and nutrient availability, as well

as high metal concentrations (Anand *et al.*, 2006). They offer the advantage of having cell wall material which shows excellent metal- binding properties (Gupta *et al.*, 2000).Amylolytic enzymes of *Aspergillus terreus*, *A.oryzyae*, *Penicillium* sp., *Fusarium moniliformae.*The present investigation was undertaken to study the factors affecting growth and amylase production by the two mold *Aspergillus* sp. and *Penicillium* sp.

MATERIALS AND METHODS

Media and growth condition

The nutrient media used for the selective isolation of fungi from soil was Potato Dextrose Agar media (PDA) containing per litre potato (peeled and sliced) 200g, Dextrose 20.0g, Agar 15.0g, pH 6.8. The Slants of PDA were used for the preservation of strains. The most favourable temperature for luxuriant growth of the organism was found to be 28±2°C for 48 hours.

Isolation of fungi from soil

One gram of soil samples was dissolved in 10 ml of sterile distilled water, shaken vigorously, diluted in 1 N saline and 0.1 ml of suitably dilute sample was plated over the surface of PDA medium.

Colonies appearing after 3 days of incubation at 28±2°C were visually classified into four morphotypes. Altogether 102 colonies henceforth assigned the isolation numbers were selected, purified by single colony isolation and the clonal cultures were used for further investigation.

Screening and identification of the organisms

Fresh culture of the isolate was grown on PDA plates and incubated at 28±2°C for 3 days Lugol's iodine was poured on full grown culture. A pale white zone against deep blue colour thus observed was measured by the method described earlier to screen for amylase producing strains (Teodoro *et al.*, 2000). The strain number AB 55 showing high level of amylase production was finally selected for further investigation. The microscopic examination was carried out using Olympus CX41 research microscope.

Culture condition for enzyme production and preparation of the enzyme

For enzyme production, the optimized media (pH 7.0) used contained per litre (1! w/v)starch, 3g $NaNO_3$, 0.5 g K_2HPO_4,1gKCLand traces of $ZnSO_4$, $MnSO_4$,$FeSO_4.7H_2O$ and $CaCl_2$. Broth cultures were raised using 10^6 spores of the organism in 50 ml of media in 250 ml.Erlenmeyer's flasks in shake culture at 28 ± 2°C and 200rpm .The supernatant of the culture filtrates obtained after centrifugation at 12000 rpm for 15 minutes at 4 °C was used for determining the extra cellular amylase activity (Lin *et al*.,1998).

Determination of amylase activity

The amylase activity was measured spectrophotometrically after addition of iodine to the substrate enzyme mixture according to the method described by Palanivelu (2001). One unit of amylase activity was defined as the amount of enzyme that produced 1 µg glucose equivalent per minute under the experimental conditions.

Protein assay

The extra cellular protein was assayed according to modified Lowry's method (1951).

RESULTS AND DISCUSSION

Altogether 102 isolates of fungi selected using the soil samples were screened for amylase production. One of these isolates (AB 55) showed higher amylase activity in bioassay plates was subjected to detailed investigations.

The microphotograph of *Aspergillus fumigatus* having chains of conidia up to 400µ, globose and dark green are visible clearly under X400 magnification.

The morphological and physiological characteristics of the strain are summarized in Table 1.According to Gilman manual the strain is characterised as *Aspergillus terreus*.

Table 2 shows the results obtained with the optimization of conditions for maximizing the amylase activity. A value of 214.2U/100 ml of enzyme activity was observed just after 24 hours of incubation and a peak value of 589.6 U/100 ml observed after 96 hours of incubation the enzyme activity dropped suddenly after 96 hours. The pH the culture filtrate was dropped from 7.0 to 4.5 after 24 hours in shaking conditions. But gradually increased up to 6.8 after 72 hours. A similar trend of initial decrease and subsequent increase in the pH of the culture media has been reported earlier in case of *Streptomyces rimosus*(Yang et al., 1999).According to Duran Paranco *et al.*, (2000),a amylase were less active under alkaline conditions

but show their optimum activity at pH 4.0 in fungi. One possible explanation of such pH changes during the growth of amylolytic micro organisms might be due to accumulation of organic acid and the residue of sulphate ion during utilization of $MgSO_4$. A similar explanation for the pH drop has been given by Yang and Wang (1999) in case of *S.rimosus*. The total protein content also increased as cultures became older up to 312µg/ml at 96 h (Table 2).

For determining the pH optima of amylase activity the sodium-potassium phosphate buffer was prepared from 4.0 to 10.0. The enzyme activity increased gradually with increase in the pH (Fig.1) and a maximum amylase activity 589 U/100 ml.was observed at the pH 7.0. However a rapid declination in the enzyme activity started at ad beyond pH 8.0. a near analogous finding were made earlier with *Streptomyces awroficiens* 77 (Shatta *et al.*, 1990) and Bacillus sp. K-12 (Kiran 2005).The amylase activity was further studied the temperature range of 30°C to 60°C for determining the optimum temperature of the enzyme action in vitro. The results presented in Fig. 3 shows that the amylase activity increased gradually along with the increase in temperature up to 50°C but suddenly dropped at 60° c and the maxim um activity is 590U/100 ml was found (Fig. 2) of activity from both (the fungi and bacteria) was however observed at 50°C. Narang and Satyanarayana (2001) and fitter *et al.*, 2001 and Lin *et al.*, (1999) have also reported that some of the a amylase work better at high temperature. The strain (AB 50) *Aspergillus fumigatus* was further characterized on the basis of its growth in presence of different metal ions. The strain showed good growth in presence of Fe^{+2},Ca^{+2},$Mg^{2+,}$ Co^{+2} up to a concentration of 5 mM .Most of amylases are known to be metal ion dependent enzymes namely divalent ions like Ca^{+2},Mg^{+2},Mn^{+2},Zn^{+2},Fe^{+2} etc(A.Pandey *et al.*, 2000). Ca^{+2} was reported to increase

α-amylase activity of an alkaliphilic *Bacillus* sp.ANT-6 (Burhan *et al.*, 2003) The metal ions showed remarkable effect on the enzymatic activities on the basis of yield of reducing sugar. However, ions like Hg^{+2}, Mn^{+2}, Zn^{+2} remarkable decreased the activity of amylase enzymes. The result presented in Fig. 4.Inhibitoryeffectof these ions in the system (Sen, S., Chakarbaty, 1984).

Table 1. Physiological characteristics of *Aspergillus terreus*

Temperature	Physiological charecterstiics				
(°C)	Growth	pH	Growth	Nacl(%)	Growth
15	-	4.0	-	2	-
25	+	5.0	+	4	+
30	+	6.0	+	5	+
37	+	6.8	+	7	+
42	+	8.0	+	10	-
50	-	9.0	+	12	-

Table 2. Biochemical characterizations of the cultutre filtrate of *A. terreus* at different time interval

Hours	pH	Protein (µgml^{-1})	Amylase activity (U/100ml)
0	7.0	0 ± 0 .00	0 ± 0.00
24	6.2	92	214.2
48	5.8	110	241.4
72	5.2	220	272.5
96	4.8	312	589.6
120	4.2	128	402.1

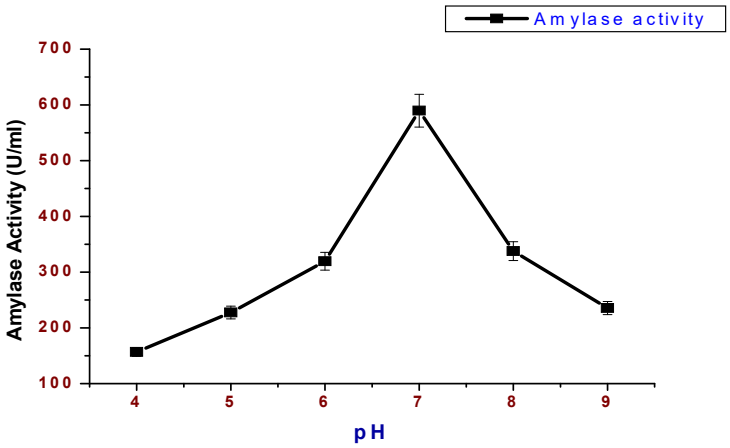

Fig. 1. Effect of pH on the extra-cellular amylase activity of *Aspergillus terreus*

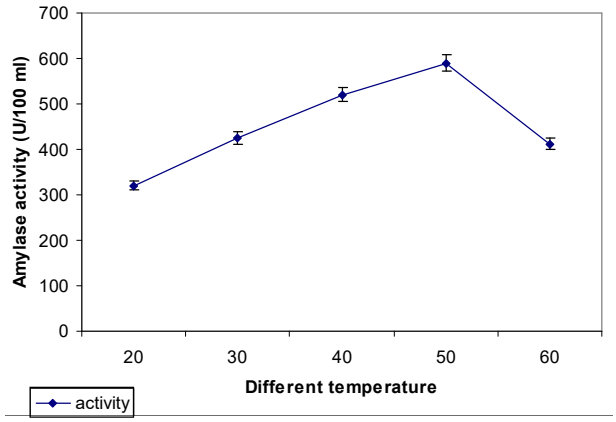

Fig. 2. Effect of temperature on the extracellular amylase activity of *Aspergillus terreus*

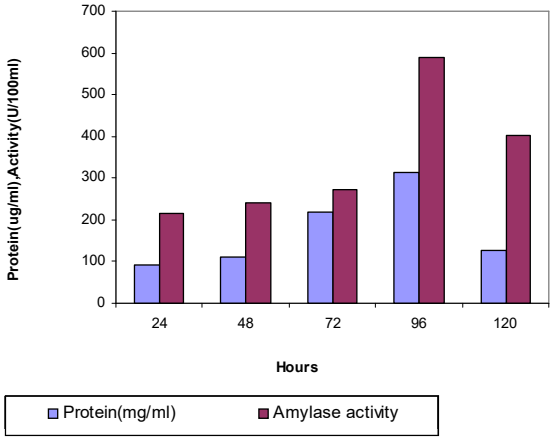

Fig. 3. Effect of different time period on the extracellular amylase activity of *Aspergillus terreus*

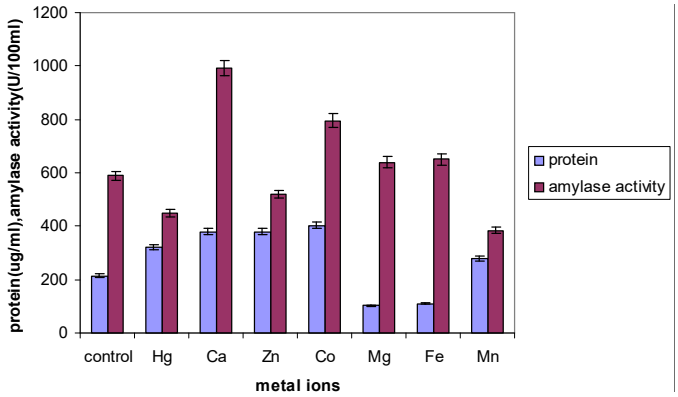

Fig. 4. Effect of different metal ion on the extracellular amylase activity of ***Aspergillus terreus***

CONCLUSION

Metal appear to affect organic biodegradation through impacting both the physiology and ecology of organic degrading micro organisms. Metal may inhibit pollutant biodegradation through interaction with enzyme involved in general metabolism.

With these rationales the attempts have been made for degradation of metal contaminated starchy waste material using micro organisms. It can be concluded that *Aspergillus fumigatus* can be industrially exploited for the synthesis of amylase and strain improvement studies can be carried out to enhance enzyme production.

ACKNOWLEDGMENTS

Authors are thankful to Dr.B.Prasad, Patna University,Patna for providing necessary facilities to conduct the experiments.

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