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IMPACT OF METAL IONS ON THE DEGRADATION OF AGROWASTE BY *ASPERGILLUS FUMIGATUS*

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A total of 102 strains of fungi isolated from soil samples in and around Patna. Three strains (AB50, AB55, AB109) showing amylase activity were screened for further investigation. Based on morpho-physiological characteristics the strain AB50 showing hyper-amylase activity was identified as *Aspergillus fumigatus*. 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 at pH 7.0 and 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.

Key words: Amylase production/activity, *Aspergillus fumigatus*, Metal ions

Starch is the primary storage polysaccharide of plants and is degraded by amylolytic enzymes produced naturally by numerous microorganisms (Lin and Hsu 1997; Kiran *et al*. 2005). Amylases from plants, animals and microorganisms have been studied in great details ever since its first discovery (Boyer and Ingle, 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). Contamination of sediments and natural aquatic receptors with heavy metals is a major environmental problem all over the world (Baldrian and Gabriel 2002, Gavrilesca 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 microbiota (Verma *et al.* 2001). Generally the contaminated sites are the sources of metal resistant microorganisms (Gadd 1993). In naturally polluted environments, the microorganism response to heavy metals toxicity depends on the concentration and on the action of factors such as the types of metal, the nature of the medium and microbial species (Hassen *et al*. 1998). Fungi and yeast biomasses are known to tolerate heavy metals (Baldrian 2003, Gavrilesca 2004). They are the versatile group, as they can adapt 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). With these rationales the attempts have been made for

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Table1. Physiological characteristics of *Aspergillus fumigatus*

Temperature (°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	-

degradation of metal contaminated starchy agro-waste material using microorganisms. The present investigation was undertaken to study the factors affecting growth and amylase production by *Aspergillus fumigatus*.

MATERIAL AND METHODS

Site of sample collection

The soil samples were collected from different sites of Patna, Bihar. For isolation of fungi, experimental pots were also prepared by adding different agro-wastes (rotten vegetables, bagasse, rice straw, corncob etc.) in soil. After one month, soil of these experimental pots was used as sample for isolation of fungi.

Media and growth condition

The nutrient media used for the selective isolation of fungi from soil was Potato- Dextrose Agar media (PDA) supplemented with Streptomycin antibiotic (200µg/ml of media). The slants of PDA were used for the preservation of fungal isolates. For colony characterization, Czapek's agar was used. The optimum temperature for incubation of culture was 28±2^{0}C for 72 hours.

Isolation of fungi from soil

One gram of soil sample was suspended in 9.9ml of sterile normal saline (0.87% NaCl, w/v), shaken vigorously, serially diluted in normal saline and 0.1 ml of suitably diluted soil suspension was plated over the surface of PDA medium.

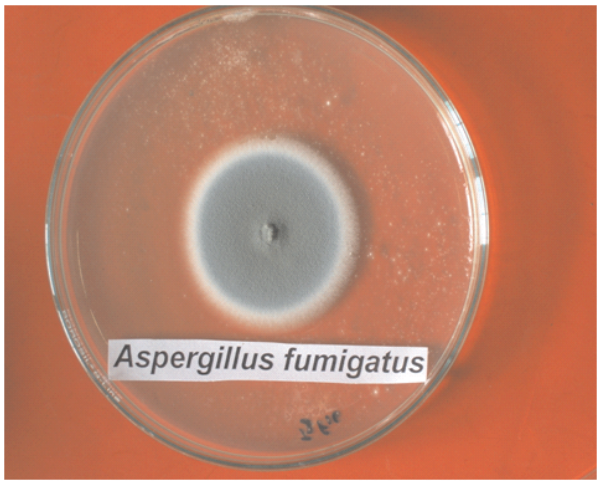

Figure 1. Single colony of *Aspergillus fumigatus* on Czapek's agar

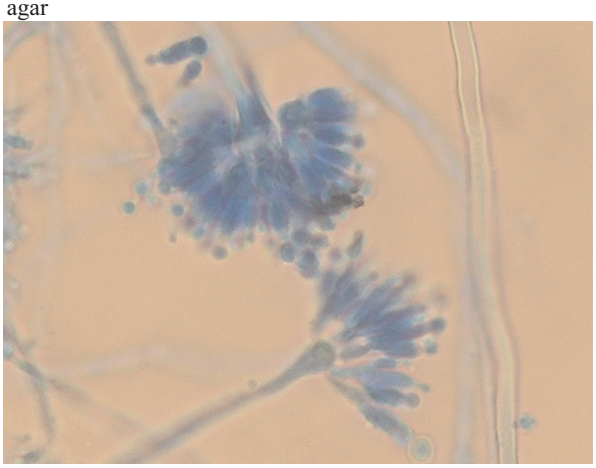

Figure 2. Microphotograph of *Aspergillus fumigatus* (Magnification x400)

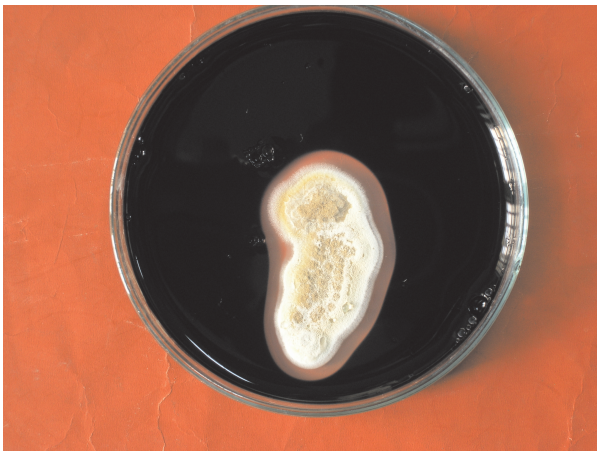

Figure 3. Starch agar plate showing positive amylase activity by *Aspergillus fumigatus*

Colonies appearing after 3 days of incubation at 28±2°C were visually classified into different morphotypes. Different morphotypes, henceforth assigned the isolation numbers,

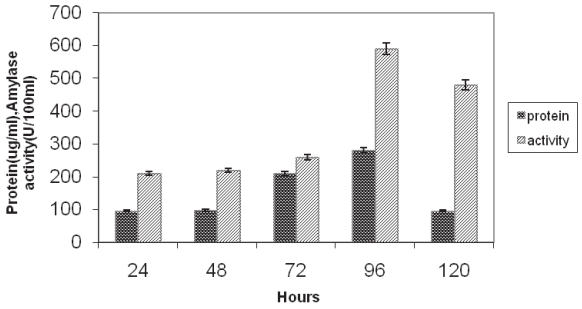

Figure 4. Effect of different time period on the extracellular amylase activity of *Aspergillus fumigatus*

were selected, purified by streak plate method. The purified selected isolate was used for further investigation (Fig.1).

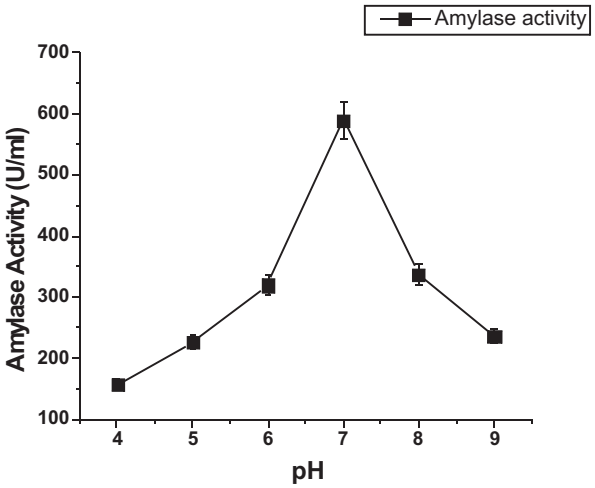

Figure 5. Effect of pH on the extra-cellular amylase activity of *Aspergillus fumigatus*

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 after that 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 and Martins, 2000). The microscopic examination was carried out using Olympus CX41 research microscope. The isolates were identified by standard manuals (Barnett and Hunter 1972, Gilman 1975).

Culture condition for enzyme production and preparation of the enzyme

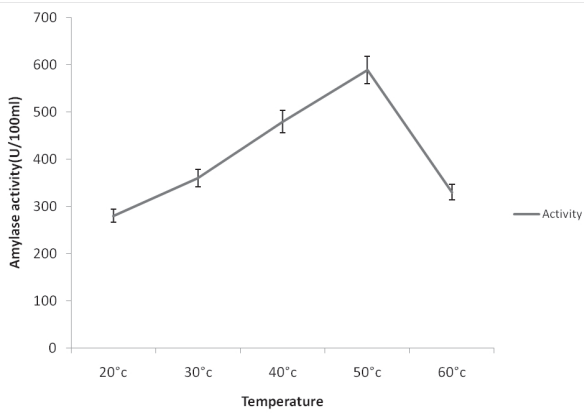

Figure 6. Effect of temperature on the extra-cellular amylase activity of *Aspergillus fumigatus*

For enzyme production, the optimized media (pH 7.0) used contained per litre (1% w/v) starch, 3g $NaNO_3$, 0.5g K_2HPO_4,1g KCL and 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 250ml Erlenmeyer's flasks at 28 ± 2°C under shake culture condition (200rpm). The supernatant of the culture filtrate 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).

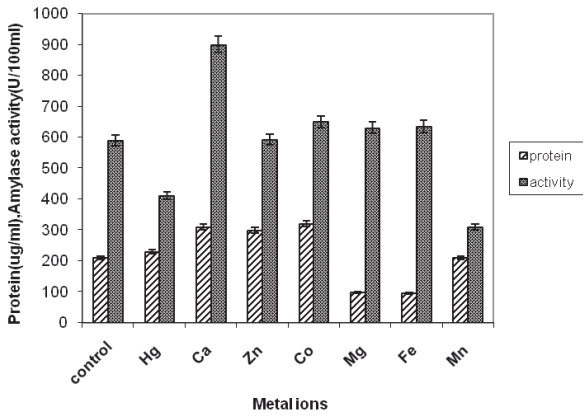

Figure. 7. Effect of different metal ions on the extracellular amylase activity of *Aspergillus fumigatus*

Determination of amylase activity

The amylase activity was measured spectrophotometrically (Hitachi-U2310) 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 were screened for amylase production. One of these isolate (AB 50) showing higher amylase activity in bioassay plate was subjected to detailed investigations (Fig 3). On the basis of cultural characteristics and microscopic morphology the isolate AB 50 was identified as *Aspergillus fumigatus* (Fig 1 and 2).

The strain AB 50 was optimized for amylase activity. The results are shown in (Fig 4, 5, 6). A value of 214.2 U/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 (Fig 4). The pH of 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 and Wang 1999). According to Duran Paranco *et al*. (2000), amylase was 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 microorganisms 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 (up to 312µg/ml at 96 hours) as cultures became older (Figure4).

For determining the pH optima of amylase activity, the sodium-potassium phosphate buffer was prepared from pH 4.0 to 10.0. The enzyme activity increased gradually with increase in the pH (Fig 5) 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 and beyond pH 8.0. A near analogous findings were made earlier with *Streptomyces aureofaciens* 77 (Shatta *et al*. 1990) and *Bacillus* sp. K-12 (Kiran 2005). The amylase activity was further studied between temperature ranges of 30°C to 60°C to know the optimum temperature for the enzyme activity *in vitro*. The amylase activity increased gradually (590 U/100 ml) along with the increase in temperature up to 50°C but suddenly dropped at 60°C (Fig 6). Narang and Satyanarayana (2001), Fitter *et al*. (2001) and Lin *et al*. (1998) have also reported that some of the amylase work better at high temperature.

The strain *Aspergillus fumigatus* AB 50 was further characterized on the basis of its growth in presence of different metal ions. The metal ions showed remarkable effect on the enzymatic activities on the basis of yield of reducing sugar. The strain showed good growth in presence of Fe^{+2}, Ca^{+2}, $Mg^{2+,}$ Co^{+2} up to a concentration of 5 mM (Fig 7). Most of amylases have been known to be influenced by metal ions like Ca^{+2}, Mg^{+2}, Mn^{+2}, Zn^{+2}, Fe^{+2} etc. (Pandey *et al*. 2000). Ca^{+2} was reported to increase α-amylase activity in alkalophilic *Bacillus* sp. ANT-6 (Burhan *et al*. 2003) However, ions like Hg^{+2}, Mn^{+2}, Zn^{+2} remarkably decreased the activity of amylase.

CONCLUSION

Metals appear to affect organic biodegradation through impacting both the physiology and ecology of organic matter degrading microorganisms. Metal may inhibit pollutant biodegradation through interaction with enzyme involved in general metabolism. It can

be concluded that *Aspergillus fumigatus* can be commercially exploited for degradation of metal contaminated starch rich agro-wastes.

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