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Effect of metal ions on amylase production

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Abstract
Amylase producing fungi were isolated from different soil samples. About 11 fungal isolates were isolated which were screened for Amylase production on starch agar medium using iodine test. Of the 11 isolates, three isolates showed clear zones and the isolate showing maximum clearance zone was selected for further studies. Identification of isolate showed that it belonged to Genus *Aspergillus*. The effect of different metal ions on the production and activity of Amylase was studied. Five different ions were used for the study. Starch agar medium was prepared with different metal ions and inoculated with the culture and incubated. It was seen that maximum mycelial growth was observed with $MgSO^4$ followed by KNO^3 but very less or negligible growth was seen when grown with $CaCO^3$. Therefore, Mg ions were considered to be the best ion for optimum growth of the fungus and thus considered the best inducer for Amylase production as maximum enzyme activity was observed with Mg ions but Ca and Cu ions showed less activity and therefore might be showing inhibitory effect on Amylase production.

Keywords: *Apergillus* and Amylase

INTRODUCTION

Many enzymes which have been exploited till date for Industrial purposes are mainly extra cellular hydrolytic enzymes, which degrade naturally occurring polymers such as starch, proteins, pectin cellulose etc. Enzymes are basically biocatalysts which catalyze a wide variety of chemical reactions. Most of the enzymes are derived from animal and plant sources, but the major source is the microbial source (Lin et al, 1997). Many Microorganisms such as yeasts, fungi, bacteria, are regarded as the sources for various enzymes for commercial applications. Fungi are the most preferred organisms for the production of various economically important enzymes owing to their high yield. Amylases are Starch-degrading enzymes which are significant in various industries ranging from food, textile to paper industries (Vyas and Dixit, 2006). The amylases can be derived from several sources such as plants, animals and microbes. The high industrial demands for amylases are met by microorganisms as they are economical (Lonsane and Ramesh, 1990). Amylases have been derived from several fungi, yeasts, bacteria and actinomycetes, however, amylase enzymes from fungi are the most dominating (Harihara and satya priya, 2012).

Amylases are responsible for the breaking of α-1, 4 glycosidic bonds in polysaccharides to release units of D- glucose. Amylolytic enzymes are of industrial importance particularly in the food and detergent industries (Kashyap *et al,* 2002) .Many fungi such as *Aspergillus niger, Penicillum, Cephalosporium, Neurospora* and *Rhizopus* are the major amylase producing microorganisms. (Pandey A, 2003).

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The present study is mainly focused on the isolation of potent, indigenous amylase producing fungi from soil and to see the effect of various metal ions on the activity of the enzyme.

MATERIALS AND METHODS

Collection of soil: In the present study, soil samples were collected from various sites like garden, agricultural land etc. Samples were dried at room temperature for 2-3 days.

Isolation of Amylase Producing Microorganisms: Serial dilution was carried out from 10^{-1} to 10^{-5} and was plated on Czapek Dox agar by spreading 0.1ml of the diluted sample. Then the plates were kept for incubation at room temperature for growth of amylase producing organisms.

Amylase Activity: The isolated fungal strains obtained after incubation for 3-5 days were sub cultured till purity and the amylase production was observed on starch agar plates (peptone 5 g, beef extract 3.0 g, soluble starch 2.0 g, agar 15.0 g and distilled water 1000 ml) by flooding with iodine solution. The amylolytic activity was confirmed by the formation of clear zone around the fungal growth (Balkan and Ertan, 2007)

Morphological Characteristics: The potent fungal strain was morphologically characterized by lacto phenol cotton blue staining.

Amylase Enzyme Assay
Separation of mycelium: The fungal mycelia mat was separated by filtration using whattman filter paper and filtrate used as crude enzyme extract.

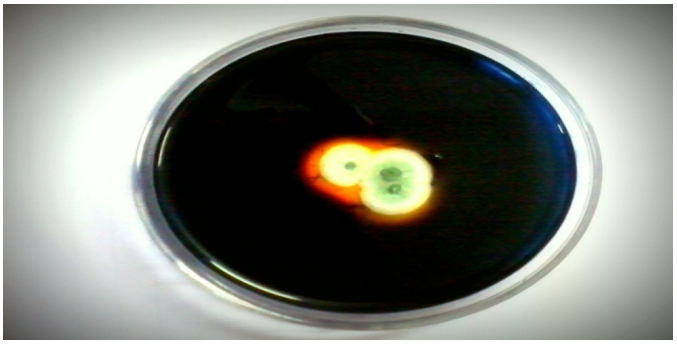
Amylase Assay: Amylase activity was assayed as described by Gupta *et al*, 2008. The reaction mixture consisted of 1ml crude enzyme solution and 2 ml of 1% soluble starch in sodium phosphate buffer of pH 6.8. The mixture was incubated for 30 min at 50°C. The

liberated level of reducing sugars in the form of glucose equivalents was determined by dinitrosalicylic acid (DNS) method of Miller (1959).The blank contained 2ml substrate and 1ml distilled water. One unit (IU) of α-amylase is defined as the amount of enzyme releasing 1µmole glucose equivalents per minute under assay conditions.

Effect of metal ions on amylase activity: Various metal ions were added to the standard assay mixture and activity of the enzyme was measured in the same way as mentioned above.

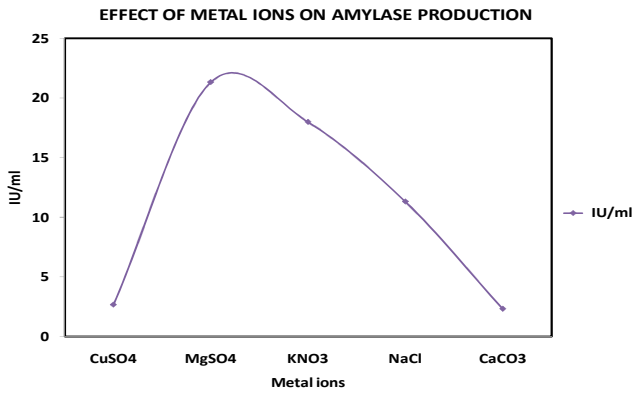
RESULTS AND DISCUSSION

Screening and identification of amylase producing fungus: 11 fungal isolates from various soil samples collected were evaluated for amylase production of which the most potent amylase producer showed a clear zone of 1.8 cm from the edge of the fungal growth. This upon staining was identified to belong to genus *Aspergillus.* This was selected for further study.



Effect of metal ions

In the enzyme action, metallic cofactors are important because their presence or absence regulates enzyme activity. The presence of specific metallic ions along with essential nutrient source can inhibit or enhance amylase activity. The effect of various metals ions were evaluated and it was observed that confluent mycelial mat growth was seen in presence of Mg^{2+} and K^{+} but little or no growth with Cu^{2+} and Ca^{2+}. Nominal growth was seen with Na^{2+}.



Also to confirm these results the enzyme activity was determined which confirmed the above results. Maximum activity of 21.33IU/ml was obtained with Mg^{2+} followed by K^{+} showing an activity of 17.9 IU/ml.These results are in confirmation with the results of Lonsane and Ramesh (1990) who also reported maximum activity and growth with Mg^{2+}. Gupta *et al* (2008) also reported maximum amylase activity in presence of Mg^{2+} followed by Na 2+ and reported inactivation with $Cu2^{+}$ and Ca^{2+} but in contrast Mayzaud (1980) reported enzyme inactivation with Mg^{2+} and found enhanced activity with Cu^{2+} and Na^{2+}.

CONCLUSION

It can be concluded that soil being a rich source of many hydrolytic enzymes can be exploited to isolate many potent indigenous microorganisms. The genus *Aspergillus*; no doubt produces a wide range of economically important enzymes including amylases. It appears that metal ions play a very important role in the growth and production of amylases and the action of metallic ions on amylase vary from one species to other.

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