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Research Article

SCREENING & SELECTION OF SOME FUNGI FOR PRODUCTION OF EXTRACELLULAR AMYLASE

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ABSTRACT
This paper represents screening and selection of fungi isolates for their extracellular enzymatic activity. Production of extracellular enzymes was investigated by employing our 66 laboratory isolates. Screening of amylase was done. Major number of isolates showed presence of amylolytic activity. Biochemical estimation for amylase activity showed 9% of the total culture isolates yielding higher production of amylase. Fungal isolate number 6 & 8 showing highest yield were selected as innoculum for optimization of media. Variation in starch content, pH and Incubation period keeping the temperature unaltered. Highest production of amylase was seen in 3% (Isolate number 6) & 4% (Isolate number 8) of starch in the media. Optimum pH was 4.5 (6) and 8.5 (8) yielding 534.13 unit/g biomass and 1269.33 unit/g biomass. 10 Days and 4 days incubation for fungal isolate 6 & 8 resulted 520 unit/g biomass, 197.62 unit/g biomass. Thus representing the high tolerance adaptability of the strains with high amylase production.

Key Words: *Extracellular enzymes, Amylolytic, Optimization*

INTRODUCTION
Amylase is the enzyme that catalyses the breakdown of starch into sugar. Amylase is produced by a variety of living organisms, ranging from bacteria to plants & humans. Bacteria & fungi secrete amylases to the outside of their cell to carry out extracellular digestion. When they have broken down the insoluble starch, the soluble end products such as (glucose or maltose) are absorbed into their cells. We have taken fungal isolates for screening and measuring the production of amylases because it is very easy to remove the fungal mycelium from the enzyme production medium and soil fungi are a very good source of amylase production.

Many chemical transformation processes used in various industries have inherent drawbacks from a commercial and environmental point of view. In particular, a greater awareness of conservation issues has forced industries to consider alternative, cleaner methods (Rao *et al* 1998) With this regard, the use of enzymes as industrial catalyst is becoming the best option, and enzymes are gradually replacing chemical catalysts in many areas of industry (Smith 1996). Microbial enzymes are becoming increasingly important for their technical and economical advantages (Cherry *et al*, 2004). With annual growth rate of about 3.3 %, the global market for enzymes reached about $2 billion in 2004 (Sivaramakrishnan et al, 2006).The microbial amylase are the most important enzymes in present day biotechnology as they meet industrial demands and they have almost completely replaced chemical hydrolysis of starch in starch producing industry and have a great significance demand ad they hold approximately 25% of the enzyme market(Sidkey et al., 2010). Amylase as amylolytic extracellular enzyme find potential application in a number of biotechnological industrial processes such as in food, sugar, fermentation, textiles and paper industry (Rezaei et al., 2009). Recent discoveries of starch degrading enzymes have led to increase the application of amylase in various industrial processes. Amylases are the most important enzymes that take over the whole industrial enzymatic production. The enzymes are produced in a large scale whose values and uses are extremely important in various aspects and thus thanks to the helpful micro-organisms. Vegetable wastes can also be used as a good source for amylase activity (Karthick & Nirmala 2011) .

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The ability to use starch as a carbon and energy source is widely distributed among different organisms. Since this polymer is water insoluble and too large to pass across the cell membrane, biodegradation occurs extracellularly. Different kinds of enzymes are required for the conversion of the starch polymer into mono and disaccharides. Animals, plants and large variety of bacteria, filamentous fungi and Yeast possess starch-degrading enzymes to convert it in to usable forms. The properties of starch degrading enzymes however, vary with the source organism, and different organisms produce one kind or a mixture of these amylolytic enzymes (Castro *et al.*, 1992). Due to the increasing demand for enzymes in various industries, there is enormous interest in searching for enzymes suitable for application, and their cost effectiveproduction techniques (Burhan *et al* 2003).

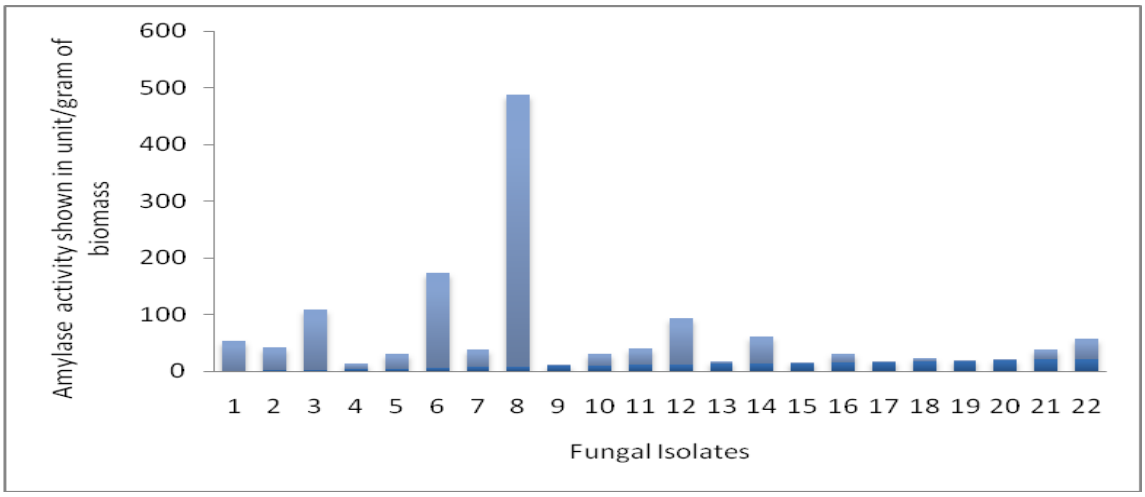

Figure1. Amylase activity (i.u. /gram) of fungi

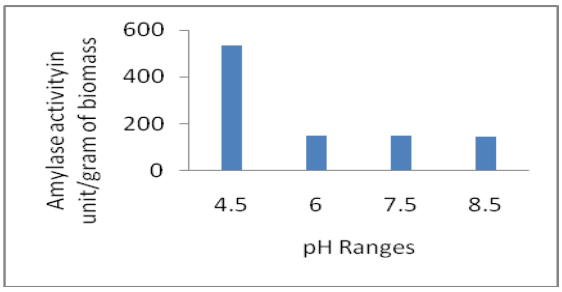

Figure 2. Effect of pH on amylase production by fungal isolate no. 6

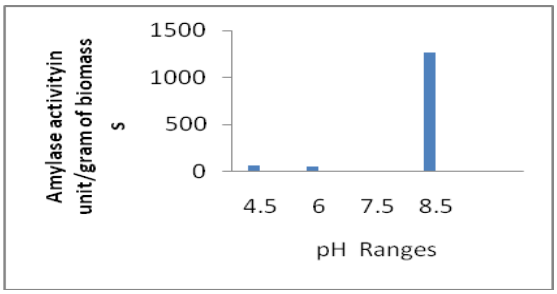

Figure 3. Effect of pH on amylase production by fungal isolate no. 8

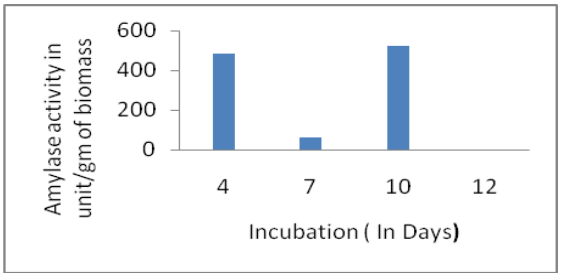

Figure 4. Effect of incubation period on amylase activity in Fungal isolate 6

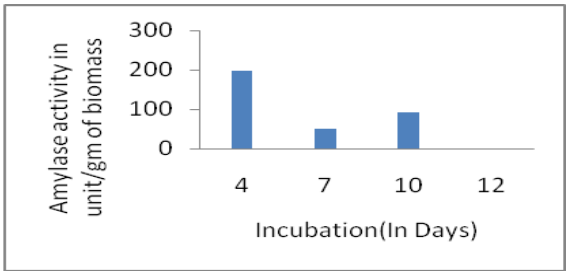

Figure. 5 Effect of incubation period on amylase activity in Fungal isolate 8

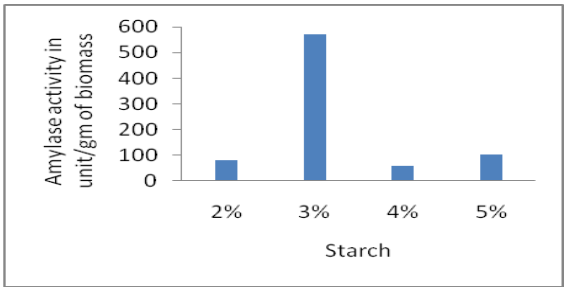

Figure. 6 Effect of starch (%) on amylase production in Fungal isolate 6

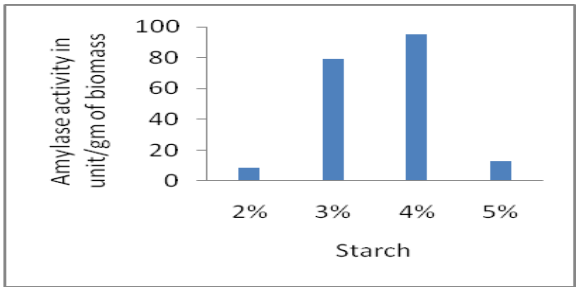

Figure. 7 Effect of starch (%) on amylase production in Fungal isolate 8

MATERIALS AND METHODS

Screening for extracellular enzymes

Cultures were obtained from Microbial culture collection of Regional Plant Resource Centre, Bhubaneswar. A total 66 culture isolates were taken for enzymatic screening for amylase activity. Isolates were grown on starch agar medium. After seven days of incubation at 28 °C the culture plates were tested. Around the fungal inoculums when Grams iodine solution (for amylase) is added the cultures making zone of clearance were selected for further biochemical estimations. Biochemical estimation for amylase was done by starch –iodine method for selected isolates showing high zone of clearance.

Amylase Assay

Starch medium with pH 6 (adjusted by 0.1 M NaOH or 0.1 N HCl) was prepared and sterilized in 150ml Erlenmeyer flask. . To the medium pure culture of the unknown fungal strain was inoculated and static incubated at 28°C for 7 days for proper growth. The cultured broth was then filtered through whatman filter paper. The fungal biomass obtained was then dried completely and weighed. The culture filtrate was used as the sample for the estimation of amylase.

To 0.05 ml 0f starch solution in a test tube 0.05 ml of phosphate buffer was added .To this 0.1 ml of sample was added and incubates at 37°c for 30 mins. At the end of this period 0.2 ml of 5% H_2SO_4 and 0.1 ml of iodine solution were added and the volume made up to 5ml. The colour is measured by spectrophotometer at 640nm against the reagent blank without starch and sample.

Optimization of Media Parameters for Profound Enzyme Activity

The factors such as Starch content, pH & incubation period affecting production of amylase were optimized by varying parameters one at a time. The experiment conducted in 200 ml of conical flask with production medium (starch content broth containing Starch (2%), Peptone (0.5%), Beef extract (0.3%). After sterilization by autoclaving the flasks were cooled and inoculated with culture and maintained under various operation conditions separately such as starch content (2% , 3 % , 4 % , 5%) , pH (4.5 ,6.0 , 7.5 , 8.5) & incubation period (4 , 7 , 10 , 12) in days keeping the temperature 28°C unaltered . The culture broth was harvested at 48 hours interval by filtering the fugal mat with wattman filter paper. A fix duration of seven days incubation was kept for first two parameters.

The culture broth was harvested at 48 hours interval by filtering the fugal mat with wattman filter paper. The supernatant was collected and used as crude enzyme solution and was assayed for enzyme activity while incubating for 4, 7, 10 and 12 days.

RESULTS AND DISCUSSION

Screening & selection of fungi by plate test method

A total of 66 isolates were screened by plate test method for amylase activity. It was observed that 9% of the total isolates showed high amylase activity , 27% are moderately potential, 34% are low potential for amylase production where as 30% of the fungal isolates does not produce amylase.

Enzyme Assay

Based on the plate test assay the amylolytic activity which was reflected by wide clear zone formation around the colony on the solid starch agar medium, 22 isolates were selected for enzymatic assay. 18% from selected isolates showed high potential of enzyme production whereas 36% resulted moderate enzyme production and the rest 45 % showed low potential for amylase. Two isolates 6 & 8 yielding 168.37 and 479.67 unit/gm of biomass respectively were selected as innoculum for optimization of media for amylase production.

Optimization of media for amylase production

***Effect of percentage of starch on amylase activity*:** The isolate number 6 showed best amylase production in 3% of starch in the medium and least production was found with 4% of starch in the medium. The highest unit/gram biomass of amylase found to be 570.90. The isolate number 8 shows best amylase production in 4% of starch in the medium and least production was found in 2% of the starch. The highest unit/gram biomass is 97.17.

Effect of variation in pH: The amylase production by isolate number 6 was best seen with the pH of 4.5 of the medium and least production was found with the pH of 8.5. The highest unit/gram of biomass is found to be 543.13. Amylase production by isolate number 8 was best seen with the pH of 8.5 of the medium and least production was found with pH 7.5 in the medium. The highest amylase produced unit/gram of biomass is found to be 1269.33.

Effect of Incubation period on amylase activity: Amylase production by fungal isolate 6 was best seen within 10 days of the incubation period. The amylase production by fungal isolate 8 was best seen within 4 days of the incubation period.

The media optimization is an important aspect to be considered in the development of fermentation technology. As there are very few reports concerning the optimization of media composition especially for fungal strains in amylase production (Quang et al ., 2000). Growth medium, pH and incubation period plays an important role in enzyme secretion by microbes. Of the 22 fungal isolates selected , 6 and 8 showed best results in various levels of starch content (3 % and 4 %) , different pH (4.5 and 8.5 respectively) and in incubation period of 10 days . As the strain was seen growing in wide range of differences in pH , this represents their adaptability and tolerance in both acidic and basic conditions thus producing large amount of enzymes unaffected . In the optimization of incubation period the actual production of amylase during growth phase of the fungal strain was known. Variation in starch content shows that the strains can accumulate good amount of starch, so the enzymatic synthesis of amylase is occuring in a productive way.

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