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Pectinolytic and Cellulolytic Activity of Soil Fungal Isolates From Similipal Bioreserve Forest

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Abstract Twenty five fungal strains were isolated from soil of Similipal Bioreserve Forest and screened for pectinase and cellulase activity. *Aspergillus sp.* was found to be predominant in the sample. Out of which, *Aspergillus niger* and *A. flavus* showed high cellulolytic and pectinolytic activity having potency index of 4.5 for I_{Fcel} and 4.1 for $I_{Fpect,}$ respectively. Parameters like pH and temperature were optimized for the potent strains. The present study concludes that both of the strains can be further utilized for large scale industrial purpose.

Keywords Aspergillus sp., cellulase, pectinase, pH, temperature

1. Introduction

In nature, microorganisms have been endowed with vast potentials. They produce an array of enzymes, which have been exploited commercially over the years. Pectinolytic and cellulolytic enzymes are known to produce by many organisms and are useful for invading host tissues. Moreover, these enzymes are essential in the decay of dead plant material by non-pathogenic microorganisms and thus assist in recycling carbon compounds in the biosphere[1].

Pectinases are important for plants as they help in cell wall extension and softening of some plant tissues during maturation and storage. They also aid in maintaining ecological balance by causing decomposition and recycling of waste plant materials. It catalyses the degradation of the pectins *via* depolymerization and de-esterification reactions [2]. Cellulase is a complex enzyme composed of cellobiohydrolases, endoglucanases and β-glucosidases which all act synergistically to convert complex carbohydrates present in lignocellulosic biomass into glucose efficiently[3]. These are inducible enzymes which are synthesized by microorganisms during their growth on cellulosic materials[4]. These are studied extensively due to their application in the hydrolysis of cellulose, the most abundant biopolymer and potential source of utilizable sugars, which serves as a raw material in the production of chemicals and fuel. Both the enzymes, pectinase and cellulase(s) are industrially important enzymes that are sold in large volumes for use in different industrial applications, for example in starch processing, animal feed production, grain alcohol fermentation, malting and brewing, extraction of fruit and vegetable juices, pulp and paper industry and textile industry[5,9].

The present investigation was based on screening of pectinolytic and cellulolytic enzyme activities of the fungal strains isolated from Similpal Bioreserve Forest soil and optimization of culture conditions of the potent isolates.

2. Materials and Methods

2.1. Study Area

Similipal Bioreserve Forest is located between 21° 35' and 22° 01' north latitude and 86° 13' and 86° 37' east longitudes. It is situated in Mayurbhanj district of Odisha state. The significance of the Bioreserve Forest is its rich biodiversity as it was protected from anthropogenic activities. Soil sample was collected from three different areas of forest, Jenabil, Bhanjabasa, and Barakanda.

2.2. Isolation and Identification of Fungi

Soil samples were collected randomly under aseptic condition from the Simipal Bioreserve Forest, Odisha. The fungal isolation from soil samples has been carried out by serial dilution method on CDA (Czapek Dox Agar) medium and the plates were incubated at 28-30℃ for 48 to 72 hours. The fungal isolates were then preserved at 3 - 4℃ for further study. The fungal isolates were examined and identified based on morphological and microscopical characteristics by following the keys by Talbot and Deacon[10,11]

2.3. Determination of Pectinolytic and Cellulolytic Activity

Fungal strains isolated were preliminary screened for pectinolytic and cellulolytic enzyme activity using disc plate method of Acuna-Arguelles et al (1994)[12]. The size

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of clearance zone formed around the colonies corresponds to the enzymatic activity of a particular culture. The cultures were individually plated on Czapek Dox Agar medium containing 1 % pectin and carboxy-methyl cellulose (CMC) as the sole carbon source. The clearance zone formed around the colonies was determined using bromothymol blue and Congo red for pectinase and cellulase activity, respectively. The potency index of cellulase (I_{Fcel}) and pectinase (I_{Fpect}) activity were calculated as the ratio of zone diameter to colony diameter. The cultures showing high potency index were further analysed for enzyme quantification study.

2.4. Pectinase and CMCase Assay

Pectinase and cellulase activity was determined following the methods of Wang *et al*[13]. One ml of the crude enzyme supernatant was incubated with 1 ml of 1% pectin and CMC in 0.1 M sodium acetate buffer solution pH 5.0 for 30 min at 63°C. The resulted reducing sugars were determined according to Miller by dinitrosalisylic acid (DNSA)[14].

2.5. Optimization of Culture Condition

The optimization for pectinase and cellulase production was performed based on the modification of the physical parameters. According to that fungal isolates were inoculated in the synthetic medium followed by incubation at different temperatures (20°C, 25°C, 30°C, 35°C, 40°C). Enzyme production was measured after 7 days of incubation by filter paper assay. Effect of pH on enzyme activity was determined by incubating enzyme (culture supernatant) in buffers of different pH (5.0, 5.5, 6.0. 6.5, 7.0)[14].

3. Result

From the result, it was observed that, the CFU/gm of the soil sample was found to be maximum in the area of Bhanjabasa ($4*10^{-4}$). A total of twenty fives species fungal strains were isolated, out of which five *Aspergillus* strains were found to have more enzymatic activity. The strains isolated were identified as *Aspergillus niger, A. terrus, A. stellatus, A. flavus, A. fumigatus*.

On the basis of potency index value, two fungal isolates, *Aspergillus niger* and *A. flavus* showed the highest potential for cellulase and pectinase enzyme activity, respectively (Figure 1).

Temperature and pH of the growth medium plays an important role by inducing morphological changes in microbes and in enzyme secretion. The cellulase enzyme activity was maximum (0.198 U/ ml of substrate) at temperature 30°C and pH 6.5 by *Aspergillus niger* (Figure 2 & 3). Similar result was also reported by Sazci *et al*. and Jahangeer [15,16]. From the figure 4 & 5 it can be concluded that the high pectinolytic activity (0.195 U/ ml of substrate) was shown by *A. flavus* at temperature 30°C and pH 6.0. According to Marcia et *al*., the activities of pectinase produced by *Bacillus sp*. strains were higher than those produced by *Aspergillus niger*, *Aspergillus sp*, *A. niger* ATCC20107, *Aureobasidium pullulans* and *Tubercularia vulgaris*[17]. The pH change observed during the growth of microbes also affects product stability in the medium[18]. At higher temperature, the organism has to spend a lot of energy for maintenance and at lower temperature transport of nutrients is hindered[19]. Hence from the result it was determined that optimum pH and temperature was different for each individual.

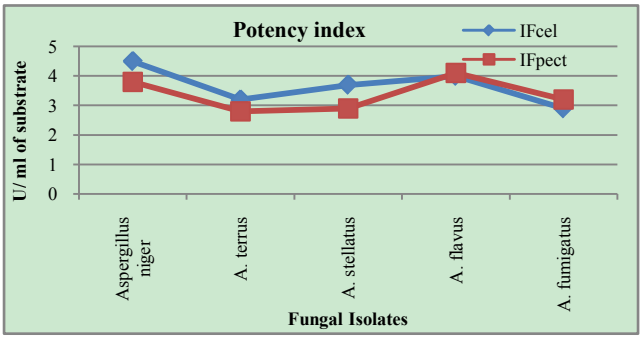

Figure 1. Potency index (I_{Fcel} and I_{Fpect}) of fungal isolates

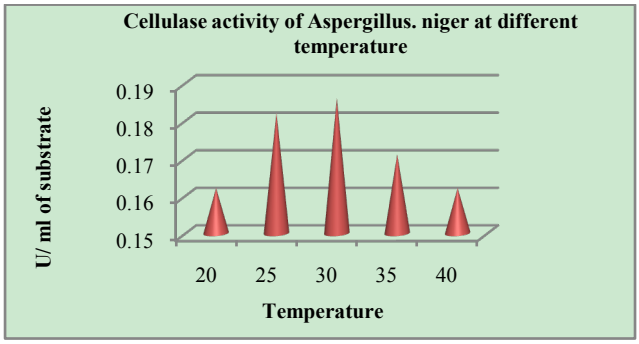

Figure 2. Cellulase activity of *Aspergillus niger* at different temperature

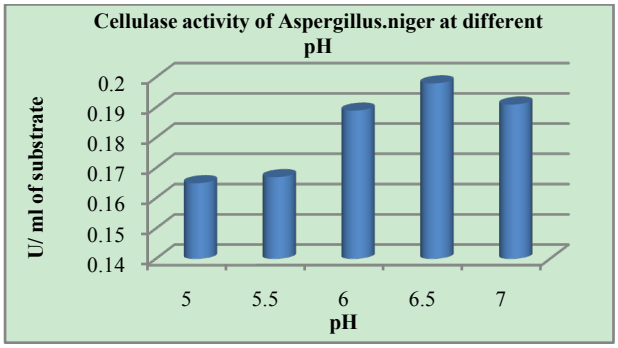

Figure 3. Cellulase activity of *Aspergillus niger* at different pH

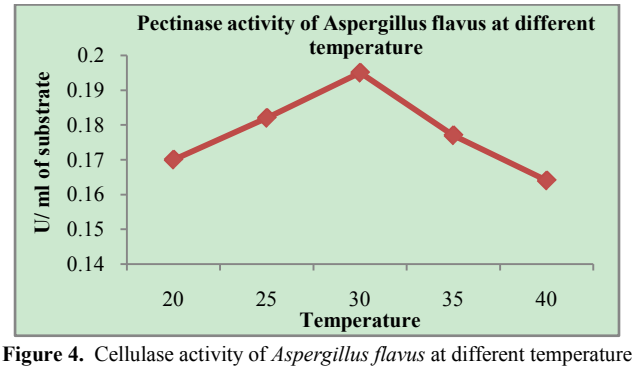

Figure 4. Cellulase activity of *Aspergillus flavus* at different temperature

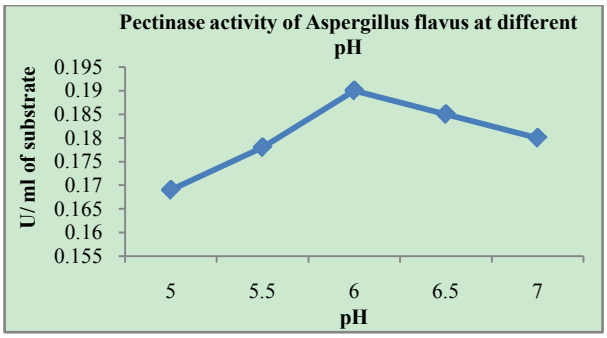

Figure 5. Cellulase activity of *Aspergillus flavus* at different pH

4. Conclusions

In nature microorganisms produce an array of enzymes which have been exploited commercially over the years. The ability to produce pectinase and cellulase by different species of *Aspergillus* has become the subject of the investigation. Maximum pectinase and cellulase enzyme activity was observed by *A. flavus* and *A. niger*, respectively. Hence a further investigation should be carried out in order to maximize the enzyme activity which will open new paradigm for industry.

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