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Exploring pectinolytic activity of *Hormodendrum cladosporioides*, a less studied fungus

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ABSTRACT

This study was designed to isolate and purify the pectinase from Hormodendrum cladosporioides, a less studied fungal isolate from the southern Western Ghats through conventional methods. This work also focuses on factors relevant for the improvement of enzymatic hydrolysis of leaf litters using that fungal strain. Different cultural conditions were examined such as pH, temperature and incubation period to assess their effect in optimizing enzyme production. The highest pectinase production was 0.394 IU/ml in the minimal medium fortified with Mangifera indica leaf litters as the carbon source. Optimum pH was found to be 5, temperature 30˚C and the 6th day was found to be the best incubation period. All the productions were done in submerged condition and incubated in a shaker at 120 rpm. After finding the suitable physicochemical parameters different nutritional supplements were incorporated to the minimal medium in different concentration for a possible enhancement of the yield. Among five of them used, peptone with 1.25 % concentration showed 0.679 IU/ml pectinase activity followed by casein which showed 0.664 IU/ml and yeast extract with 0.663 IU/ml in the same concentration. We concluded that the minimal medium fortified with M. indica leaf litter wastes with a substrate probably a nitrogen source in optimum conditions are helpful for the fungus to yield maximum enzyme production.

Key words: Pectinase, *Hormodendrum cladosporioides*, leaf litters, DNS method.

INTRODUCTION

Naturally, microorganisms have been endowed with vast potentials in which they produce an array of enzymes, which have been exploited commercially over the years. Pectin is present in highest concentration in the middle lamella of plant cell wall as a cementing substance between adjacent cells. Pectinases are important for plants as they help in cell wall extension and softening of some plant tissues during maturation and storage. Pectinolytic enzymes are known to be produced by many organisms and are useful for invading host tissues. Moreover, these enzymes are essential in the decay of dead plant materials by saprophytic microorganisms and thus assist in recycling carbon compounds in the biosphere (Alaea, 1989). 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 (Wood and Kellogg, 1988). Plant pathogens attack target cells by producing number of cell degrading enzyme which facilitates the entry and expansion of pathogen in the host tissue (Jayani *et al*., 2005).

Selection of the microbial source for pectinase production depends on several features, such as the type of production procedures (solid-state or submerged condition), number and type of the produced pectinase, pH and thermal stability of the enzymes, and genotypic characteristic of the strain (Ernesto, 2006). In our studies we selected a less studied fungus named *Hormodendrum cladosporioides*, for pectinase production. To the best of our knowledge, this organism has not been previously considered for this purpose in biotechnology.

MATERIALS AND METHODS

Qualitative analysis for pectinase activity

The fungus was tested qualitatively by growing on pectin screening agar medium (Panda *et al*., 2004) with 1 gm pectin, 0.3 gm diammonium orthophosphate, 0.2 gm KH_2PO_4, 0.3 gm K_2HPO_4, 0.01 gm $MgSO_4$ and 2.0 gm agar in 100 ml of distilled water. The pH of medium was adjusted to 4.5. This medium was sterilized and distributed aseptically in Petri dish, inoculated and incubated at 30°C. After the colony reached around 3 mm, iodine - potassium iodide solution (1.0 g iodine, 5.0 g potassium iodide and 330 ml H_2O) was added to detect zone of clearance which was the indication of pectinase.

Substrate pretreatment

Tectona grandis, *Mangifera indica* and *Artocarpus heterophylla* leaf litters were selected as carbon source in the production medium. Dried and powdered leaf litters were soaked in 1*N* NaOH (500 ml) for 24 hrs. Then excess alkali was decanted and repeatedly washed with distilled water till it reached neutral pH and then dried over night at 60ºC in a hot air oven. This product was incorporated into the medium as the carbon source for the enzyme production.

Pectinase production

For enzyme production submerged condition was maintained in 250-ml Erlenmeyer flasks by taking 100 ml of production medium in each containing (in g/l):$(NH_4)_2SO_4$ 0.1, $MgSO_4 \cdot 7H_2O$ 0.5, KH_2PO_4 0.5, and $FeSO_4 \cdot 7H_2O$ 0.0005 and pretreated leaf litters (1g /100ml). After the pH of medium was adjusted with a range of 4.0, 5.0, 6.0, 7.0 and 9.0., 100 ml of the medium was distributed in a series of flasks and sterilized at 121°C for 15 min. This was cooled and inoculated. The inoculation followed by 10 days of incubation at 20°C, 30°C, 35°C and 40°C in a shaker at 120 rpm. The samples were withdrawn on the 2^{nd}, 4^{th}, 6^{th}, 8^{th} and 10^{th} days for enzyme assays.

Extraction of pectinase enzyme

The contents of the flasks were harvested at 48 hrs intervals (two days) by adding 0.2 M acetate buffer, pH 5.5 (1:2.5 - substrate: buffer), incubated in a temperature controlled bath at 32°C for a period of 1 hr and filtered with filter paper (Whatman no. 1) under vacuum. The supernatant was used as a crude enzymatic extract and used for enzyme assay.

Optimization of pectinase with different substrates

An attempt was made to enhance the enzyme production with different substrates such as peptone, yeast extract, urea, casein and whey in different percent concentrations (0.25, 0.5, 1.0 and 1.25) at pH 5 in 30°C for 6 days of incubation period and in *M. indica* leaf litter minimal medium, i.e the best suit physicochemical parameters found. In doing so the enzyme production was increased.

Enzyme assay

Enzyme assay was based on the determination of reducing sugars produced as a result of enzymatic hydrolysis of pectin by dinitro salicylic acid (DNS) method (Miller, 1959). It is an alternative to Nelson (1944) Somogyi (1952) method. For this, to 0.2 ml of 1 % pectin solution, 2.0 ml of sodium citrate buffer of pH 5.0 and 1.0 ml of enzyme extract was added. The reaction mixture was incubated at 35°C ± 1°C for 25 min. After 25 min, 1.0 ml of this reaction mixture was withdrawn and added to test-tubes containing 0.5 ml of 1*M* sodium carbonate solution. To each test tube, 3.0 ml of DNS reagent was added and the test-tubes were shaken to mix the contents. The test tubes were heated to boiling in a boiling water-bath for 10 – 15 min, cooled and 20 ml of distilled water was added to the contents of each tube and the absorbance was measured at 570 nm using a spectrophotometer. The enzyme and substrate blanks were run parallel. A standard curve of glucose was used to calculate the reducing sugars released by the enzymatic activity. One enzyme unit of endopolygalacturonase is the number of µmol of reducing sugars measured in terms of glucose, produced as a result of the action of 1.0 ml of enzyme extract in 1 minute at 35°C ± 1°C.

$$\text{Pectin activity (U/ml)} = \frac{\text{Control-treated (unutilized pectinase)}}{\text{Enzyme concentration X incubation time}}$$

SDS - PAGE analysis

The crude pectinase enzyme preparation of *H. cladosporioides* was subjected to SDS-PAGE to determine the homogeneity and molecular weight of the enzyme. It exhibited three bands on SDS-PAGE (Fig.8). On comparing it with electrophoretic mobilities of standard molecular weight markers, it showed molecular weight of 43 kDa. The molecular weight of pectinase was determined between 36-50 kDa in the studies of Yogesh *et al*. (2009).

RESULTS AND DISCUSSION

Maximum pectinase activity was observed in *M. indica* leaf litter minimal medium (0.394 IU/ml) on the 6th day of incubation period in pH 5 at 30°C followed by 0.302 IU/ml in *A. heterophylla* leaf litters minimal medium at pH 5 and in 35°C and the least quantity (0.251 IU/ml) in *T. grandis* leaf litters minimal medium in 30°C at pH 5 (Fig 1& 5).

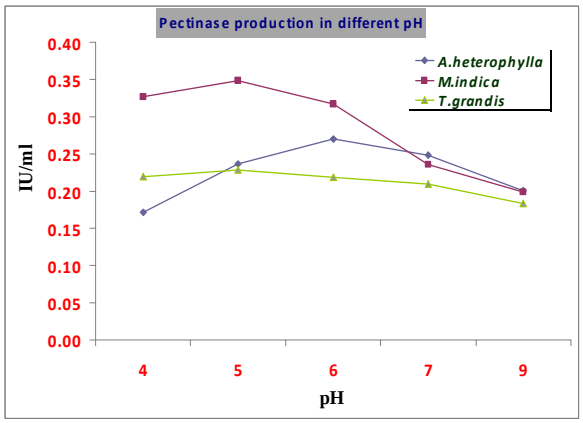

Fig. 1

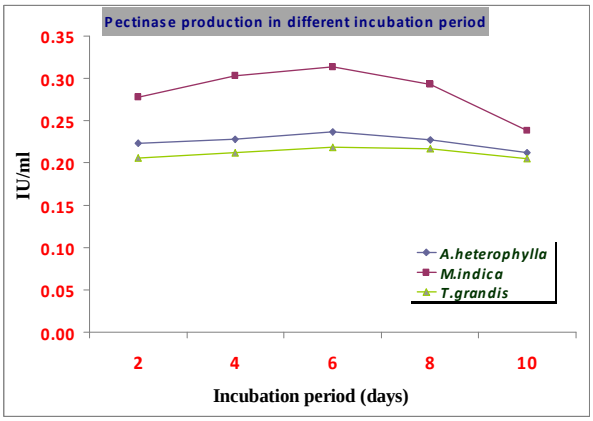

Fig. 2

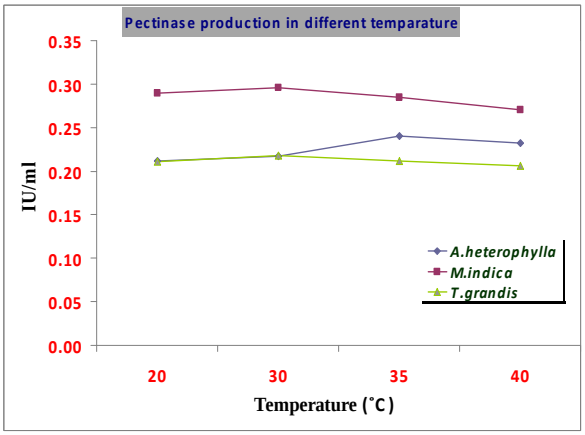

Fig. 3

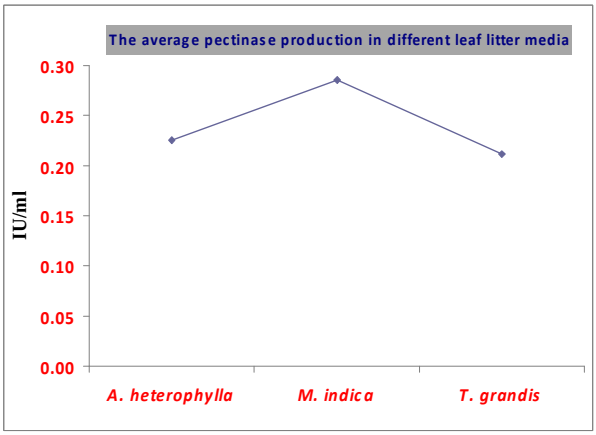

Fig. 4

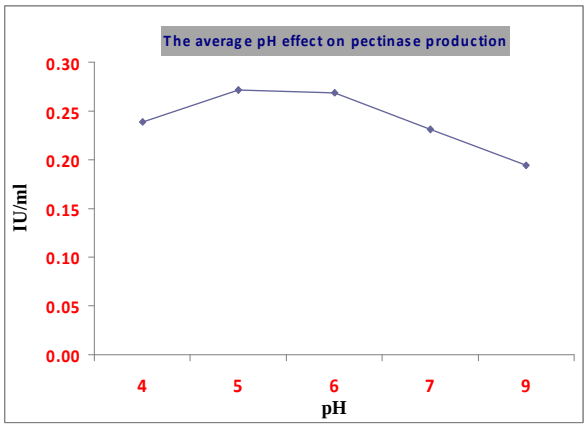

Fig. 5

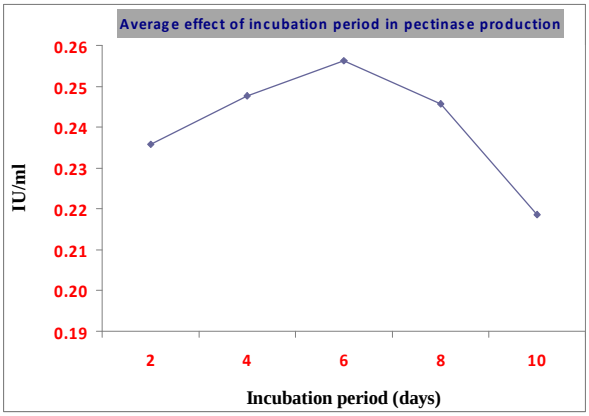

Fig. 6

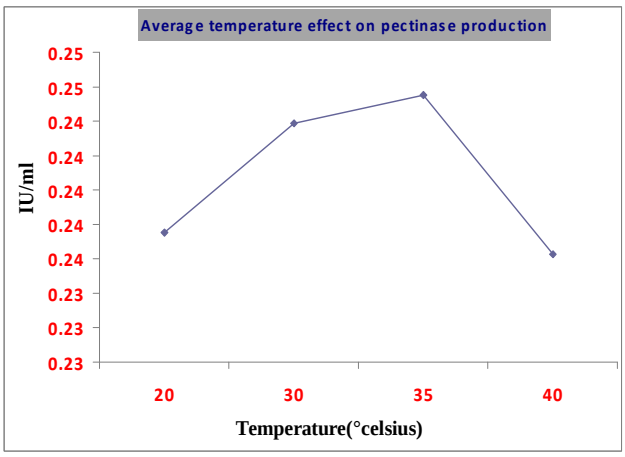

Fig. 7

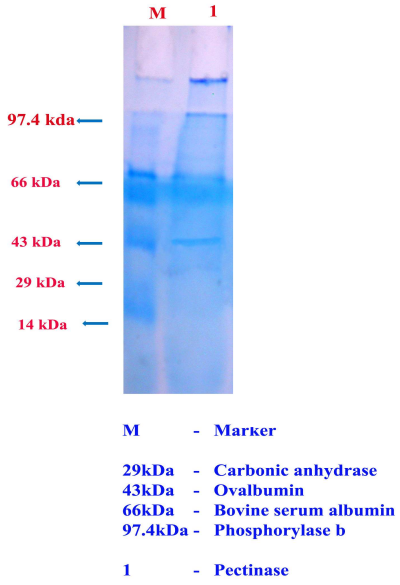

Fig. 8

The enzyme activity seemed to be increasing from the 4th day and reaches at maximum on the 6th day in the *M. indica* leaf litter medium (Fig. 2 & 6). It seemed that the organism preferred *M. indica* leaf litter medium more than *A. heterophylla* and *T. grandis* (Fig 4). When peptone, yeast extract, urea, casein and whey were employed to enhance the yield the fungus yielded 0.664 IU/ml from 0.394 IU/ml with casein in 1.25 % concentration. Maximum enzyme activity was found after 5th day of incubation and later on started depleting. The temperature range was often 30 and 35°C for enzyme activities (Fig 3 & 7). It was also reported earlier that the range of the incubation temperature for the pectinase production was 22-37°C by Hande *et al*., (2012).

Lakshminarasimha and Sreeramulu (2012) reported that the optimum temperature for enzyme production was 30°C. This shows that the pectinase producing fungi prefers room temperature for enzyme production. It was known that the period of production depends upon the nature of medium, producing organism, concentration of nutrients and the process' physiological conditions. Patil and Dayanand (2012) explained this by pointing out that the temperature is known to influence the metabolic rate of the organism involved in the process, and this determines the amount of the end product. Nevertheless, the optimum growth temperature may be different from the optimum for product formation (Bhargav, 2008).

Results of this study indicated that *M. indica* leaf litter could be an attractive and promising substrate especially in submerged fermentation for the production of pectinases by *H. cladosporioides*. It would be worth to look into the complex nutritive value of *M. indica* leaf litter for their further exploration to produce fungal pectinase.

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