

ISOLATION AND SCREENING OF PECTIN LYASE PRODUCING FUNGI FROM SOIL SAMPLE OF DEAD ORGANIC MATTERS

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ABSTRACT

Pectinolytic enzymes are one of the several extracellular enzymes produced by fungi that break down pectin, a polysaccharide substrate that is found in the cell walls of plants. Pectinases are both plant and microbial origin. Pectin lyase extensively used in the clarification of fruit juices and wines. Currently, they are widely used in industry for retting of natural fibers and extraction of oils from vegetable and citrus peels. In the present investigation, several fungi were isolated from dead and decayed organic matter of soil on pectin agar medium. The following fungal organisms *Aspergillus fumigatus*, *A. flavus*, *A. niger*, *A. ochraceus*, *A. oryzae*, *A. sydowii*, *Trichothecium sp.*, *Penicillium sp.*, *Trichoderma harzianum* and *T. viride* were screened and tested for pectinolytic activity by plate assay. Zone of clearance observed in plate

assay showed the production of pectin lyase by these fungi. The plate Assay method clearly indicated the ability of these fungi to produce pectin lyase enzymes, especially the polygalacturonase enzyme.

KEYWORDS: Pectin lyase, polygalacturonase, Pectin, *Aspergillus spp.*

INTRODUCTION

Pectin is a structural heteropolysaccharide contained in the primary cell walls of terrestrial plants. It was first isolated and described in 1825 by Henri Braconnot. Pectin lyases are the only known pectinases capable of degrading highly esterified pectins (like those found in fruits) into small molecules via β -elimination mechanism without producing methanol, in contrast with the combination of Polygalacturonase and Pectin esterase, which are normally found in commercial products.

The alkaline pectinase is inappropriate for use in the food industry due to the acidic pH of fruit juices. However, they have a very high demand in the textile industries. They are used for retting of plant fibers such as ramie, sun hemp, jute, flax and hemp. The first report on retting of sun hemp (*Crotalaria juncea*) by pectin lyase produced by *Aspergillus flavus* MTCC 7589 was published in 2008 but this aspect of pectin lyases needs to be extensively investigated further (Yadav, *et al.*, 2008).

Fungi produce several extracellular enzymes that result in the decomposition of organic matter and one such enzyme is pectinolytic enzymes. The fungus produces these enzymes to break down the middle lamella in plants so that it can extract nutrients from the plant tissues and insert fungal hyphae. The members of the fungal genus *Aspergillus* are commonly used for the production of polysaccharide degrading enzymes. This genus produces a wide spectrum of cell wall degrading enzymes, allowing not only complete degradation of the polysaccharide but also tailored modification by using specific enzymes purified from these fungi. In this study an attempt has been made to isolate and screen fungi from different sources for their ability to produce pectinolytic enzymes.

MATERIALS AND METHODS

Isolation of fungi

Isolation of pectin lyase degrading fungi was done from agricultural waste, leaf litter, and farm yard manure and cow dung by serial dilution method on pectin agar media following standard procedures. The plates were incubated at room temperature for about 3 to 5 days. The plates were observed for the growth of fungal colonies after incubation period and pure cultured.

Screening of Pectinase Activity

Pectinase activity was detected by growing fungi in a petriplate on mineral salt agar medium (NaNO₃-2.0 g, KCl-0.5 g, MgSO₄·7H₂O-0.5 g, K₂HPO₄-1.0 g, FeSO₄·7H₂O-0.01 g, Citrus pectin -10.0g, Agar-20.0g, pH-6.8-7.0, Distilled water- 1000 ml). The inoculated plates were incubated for about 72 hours at room temperature. After the colonies reached around 3 to 4 mm, potassium iodide solution was added to detect the clear zone.

Pectin Lyase Production

The isolated strain was inoculated into the sterilized fermentation medium. KH₂PO₄ - 4g , NaHPO₄ 6g , FeSO₄·7H₂O- 0.01g, CaCl₂-0.01g, MgSO₄·7H₂O-0.2g, (NH₄)₂ SO₄- 2g, H₃BO₃-

10µg/L, MnSO₄ -10µg/L, ZnSO₄·7H₂O -70 µg/L, Citrus pectin-20g, pH- 4.1 medium was incubated at 30°C for 5 days.

Enzyme Extraction

Pectin lyase was extracted from the fermented broth by a simple contact method (Krishna and Chandrasekaran, 1996). To each flask 100 ml of Tris HCl buffer of pH 8 (assay buffer) was added 2 times and each time samples were shaken (150 rpm) for 1 hour. Contents were filtered and the filtrates were centrifuged at 10,000 rpm for 10 minutes at -10°C to remove undissolved matter and fungal spores. The supernatants of two extractions were mixed and subjected to enzyme assay.

Pectin Lyase Assay

Assay of pectin lyase was performed by the method described by soares and silva (1999) 0.5 ml of enzyme was incubated for 1 h with 0.5 mL of 0.5% pectin and 1 mL of 50 mM Tris HCl buffer (pH 8) and 1 mL of 0.2 mM CaCl₂. After 1 h, absorbance was measured at 548 nm against blank solution. One unit of Pectin lyase activity was defined as the amount of enzyme present in 1 mL of original enzyme solution which released 1 µM of galacturonic acid in 1 min.

RESULTS AND DISCUSSION

Isolation of Fungi

Following fungi were isolated and identified based on cultural characteristics and sporulation. *Aspergillus fumigatus*, *A.flavus*, *A. niger*, *A.ochraceus*, *A. oryzae*, *A.sydowii*, *Trichothecium* sp., *Penicillium* sp, *Trichoderma harzianum* and *T. viride* (Fig.1).

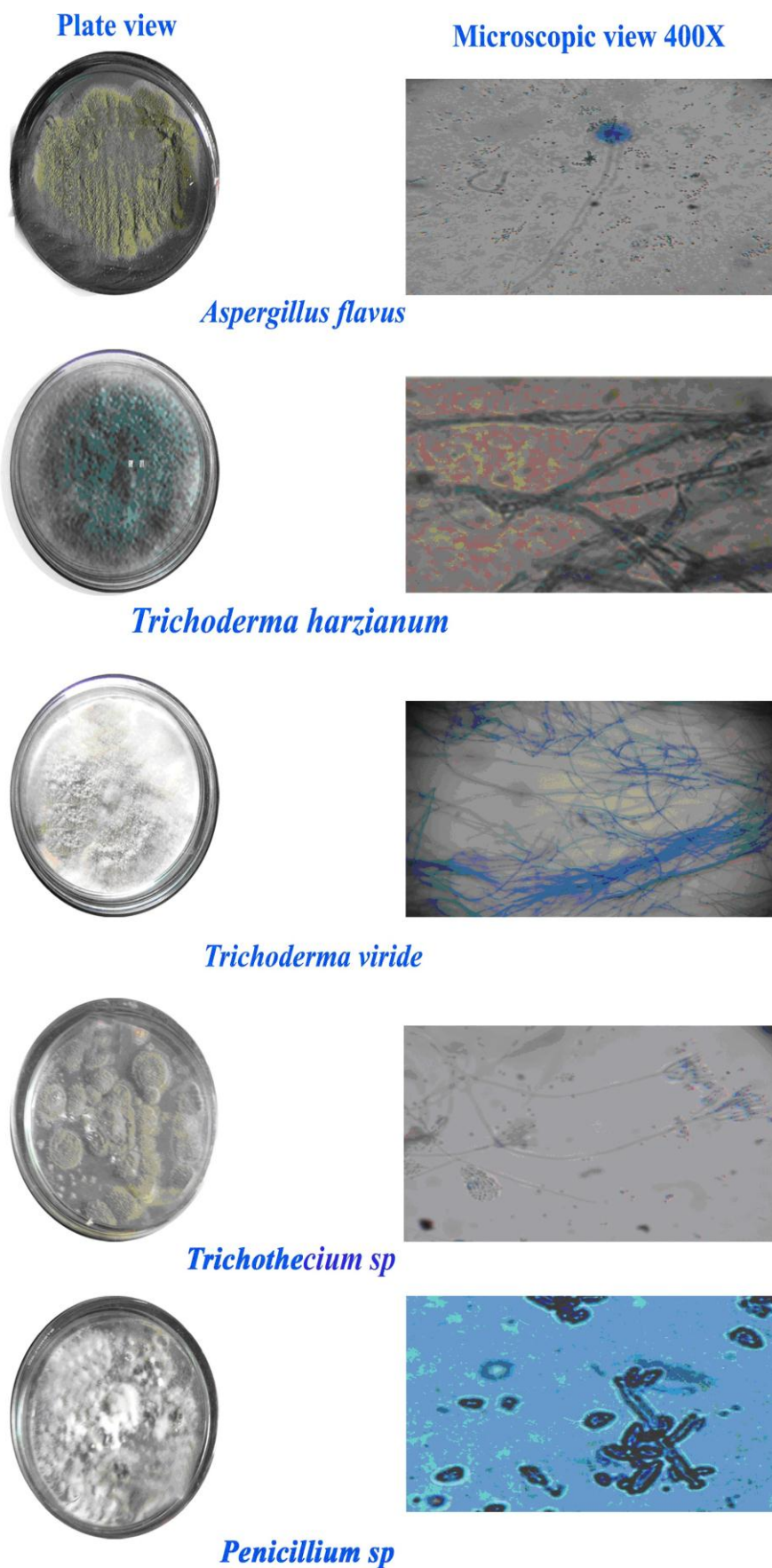
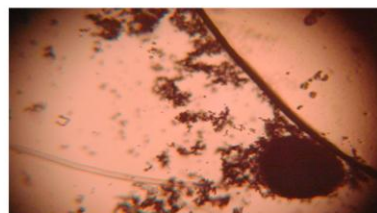
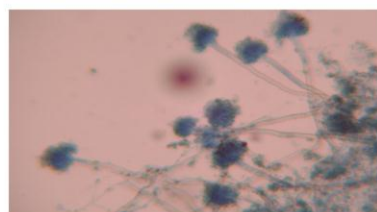
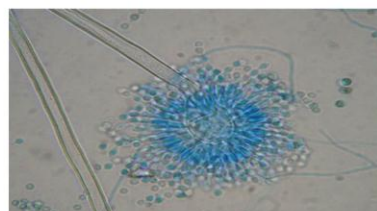
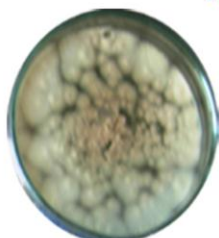
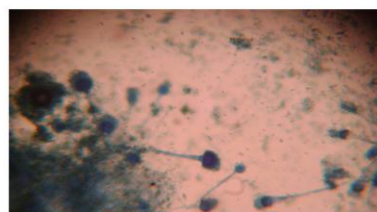
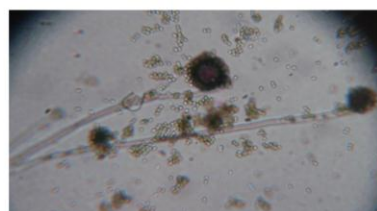


Figure 1. Isolation and Identification of fungi from dead organic matter.

Isolation and Identification of fungi from dead organic matter

Plate view

Microscopic view 400X

*Aspergillus niger**Aspergillus ochraceus**Aspergillus sydowii**Aspergillus fumigatus**Aspergillus oryzae*

Screening of Pectinolytic Activity by Plate Assay

Aspergillus flavus was able to produce pectinolytic enzyme by submerged fermentation. The enzymatic extract formed clear halos in qualitative agar well diffusion assay. All the strains were tested for pectin hydrolysis by plate assay in duplicates, at pH 7.0. Around producer

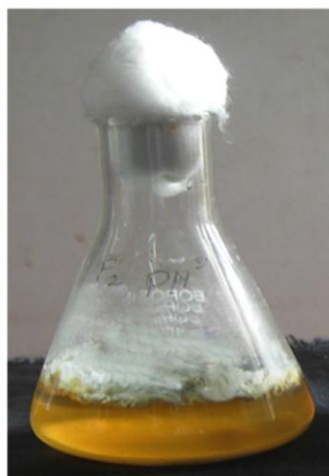
colonies, unstained areas indicated break down of pectin to galacturonic acid by cleaving the α -1,4 glycosidic bonds between two galacturonic acid residues while non-producing colonies were surrounded by opaque gel containing non degraded pectin (Fig.2).

Yadav *et al.*, (2008) reported pH 8 as optimum pH for pectin lyase production by fungi. Depending upon the fungal species, various suitable temperatures for the growth and production of pectin lyase were reported (25°C to 35°C) by mesophilic fungal species.

Screening of Pectin lyase Activity



Aspergillus flavus



Production of Pectin lyase enzyme

Figure 2. Screening and Production of Pectin lyase Activity.

Enzyme Activity

In the current investigation, maximum pectin lyase activity was observed after 48 h of incubation. With the increase in incubation period, production of enzyme decreased due to accumulation of waste material and unavailability of nutrients. Pectin lyase has maximum activity at pH 8 of the growth media, indicating that pectin lyase produced by *A. flavus* is alkaline in nature. Presence of 60% water contents other than inoculums is the most suitable

for both, fungal growth as well as pectin lyase secretion. Pectin lyase activity and total protein content in the broth during fermentation was observed and recorded. Pectin lyase production was maximum due to pectin lyase activity which was obtained after 5 days of incubation and recorded 0.20 IU/ml. The protein concentration in enzyme was determined by spectrophotometer at 610nm (Fig.2). Enhanced pectin lyase production might be related with the growth of fungi at suitable nutritional conditions. Presence of yeast extract as a carbon source and additional nitrogen sources in the growing media increased the pectic lyase production. Tween-80 interacted with pectin lyase and disrupts its 3-dimensional functional structure and made its non-functional. Peptone contains various amino acids that release nitrogen for the growth of fungi in the media, in the presence of easily available nitrogen source, the growth of fungi increased (Martin *et al.*, 2004; Margesin *et al.*, 2005).

Similar results were reported in the present study that *A. flaus* is a good source of pectin lyase production. In order to achieve huge amount of active pectin lyase sophisticated purification techniques should be needed. Better utilization of these isolated fungi requires further studies.

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