PECTINOLYTIC ACTIVITY OF *Aspergillus niger* ON PECTIC AGRICULTURAL SUBSTRATES

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ABSTRACT

Pectinase enzymes have important application in food, textile and agricultural industries. In the present study, the exopectinase and endopectinase activities of *Aspergillus niger* were investigated on two pectic substrates (wheat bran and citrus pectin). The amount of reducing sugar (D-galacturonic acid) which was released in supernatant measured for determination of exopectinase activity. Endopectinase activity was determined using viscometrical assay. The best production of the enzymes achieved in the second day for exopectinase and the sixth day for endopectinase in the cultures containing citrus pectin as the sole carbon source, in contrast to wheat bran.

Keyword: pectinase, *Aspergillus niger*, pectin, wheat bran

INTRODUCTION

Plant cell walls have primarily composed of cellulose, hemicellulose, various soluble proteins, and pectin. Pectin, a diverse family of polysaccharides, is a major structural component of the cell wall. Pectin has gelatinized and concentrated characterization that is used in different industries (Tripodo et al,2007). Pectinases play a very important role in various biological processes across the whole spectrum of life (Sprockett,2009). Pectinases have been used in several conventional industrial processes over the years, such as textile, plant fiber processing, tea and coffee industries, oil extraction, treatment of industrial

wastewater, containing pectinacious material, purification of viruses and paper manufacturing (Jayani et al,2005).

Pectinases share about 25% of global sale in the food enzymes. They are one of the most widely distributed enzymes in bacteria, fungi, and plants (Rombouts et al,1980). Pectinase production by microbes varies according to the composition of growth medium and the cultivation conditions, that is, pH, temperature, aeration, agitation, and incubation time (Thakur et al,2010).

Different fungi strains have been used to product of pectinases. Extracellular pectinases are easier and cheaper to use in great quantities. Most important applications of these enzymes are in juices and wines making, and in the processing of vegetables. Submerged or solid state mediums are used for producing of the pectinolitic enzymes by fungi (Bali,2003).

Polygalacturonases (PGases) are the pectinolytic enzymes that catalyze the hydrolytic cleavage of the polygalacturonic acid chain with the introduction of water across the oxygen bridge. The PGases involved in the hydrolysis of pectic substances are endo-PGase and exo-PGase. Polygalacturonases (pectinases) catalyze the random hydrolysis of 1→4 α-D galacturonic acid linkages in smooth region of pectin (Jayani et al,2005).

MATERIALS AND METHODS

Microorganism: The fungus *Aspergillus niger* PTCC 5013 was obtained from Iranian research organization for science and technology (IROST), and cultured on Saubarod dextrose agar (SDA) at 28±2 °C for 4 days.

Culture media: For enzyme production two substrates: citrus pectin(1% w/v), and wheat bran(1% w/v) used as the sole carbon source in a batch basal medium containing:0.6g $(NH_4)_2SO_4$, 0.6g K_2HPO_4, 0.6g KH_2PO_4 and 0.01g $MgSO_4.7H_2O$ (Okafor et al,2010) in the volume of 100 ml. A 1×1 cm piece from the center of the colonies on SDA was taken and inoculated to 100 ml of sterile submerged medium in 500 ml flasks. Fungal growth and enzyme production achieved in 28±2 °C with shaking in 100 rpm. The activity of pectinolytic enzymes were assayed in the second, fourth and sixth days after inoculation. The cultured media were centrifuged at 10000 rpm in 4 °C for 20 minutes and the supernatants were used for enzyme activity assay.

Exopectinase activity assay: Exopectinase activity was determined based on the amount of reducing sugar (D-galacturonic acid) released in culture supernatant. 1 ml of culture supernatant was added to 3 ml sodium acetate buffer (2M, PH= 5) containing 1% citrus pectin. After 10 minutes in 50 °C, the enzyme activity was stopped in 100 °C for 3 minutes. The amount of D-galacturonic acid was determined by dinitrosalicilic acid colorimetric method (Clowich,1995). The absorbance was measured at 540 nm. The unit of enzyme activity was defined as the amount of enzyme that releases 1 µmol of galacturonic acid per

minute according to the standard curve. The starndard curve was drown based on the absorbance in different concentrations (µg/ml) of D-galacturonic acid.

Endopectinase activity assay: Eendopectinase activity was assayed by using viscometrical method (Blandino. 2002). 2 ml of culture supernatant was added to 6 ml buffer containing 1% substrate. The enzyme activity was stopped in 100˚C and the mixture centrifuged at 1000 rpm. The viscosity of supernatant was determined by using Ostwald viscometer. One unit of enzyme activity was defined as the amount of enzyme that reduced the initial viscosity by 50% per minute under constant condition, in contrast to enzyme laked control.

RESULTS

Reducing sugar standard curve: The absorbances of the different concentrations of D-galacturonic acid at 540 nm are shown in figure 1.

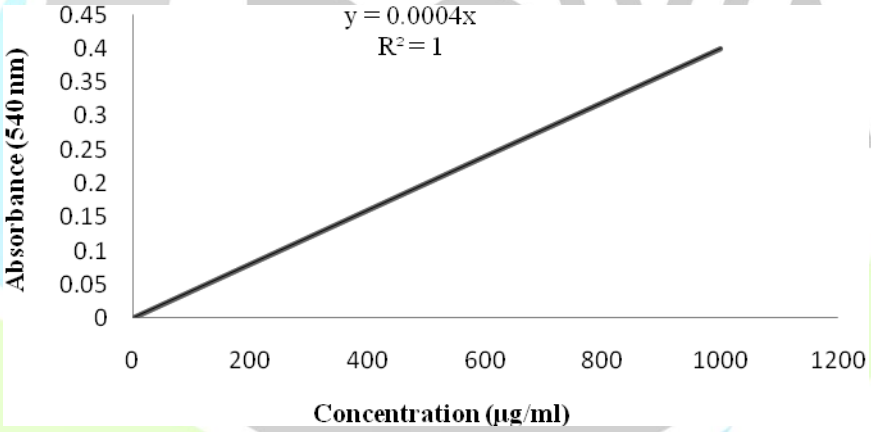

Figure 1: The standard curve of D-galacturonic acid

Daily enzyme activity: The best production of exopectinase was achieved in the second day (figure 2) and the best production of endopectinase was achieved in the sixth day (figure 3) after inoculation, in the culture condition.

The effect of substrate on enzyme production: As shown in figures 2 and 3, the enzyme production was increased by using citrus pectin as the sole carbon source, compared to wheat bran.

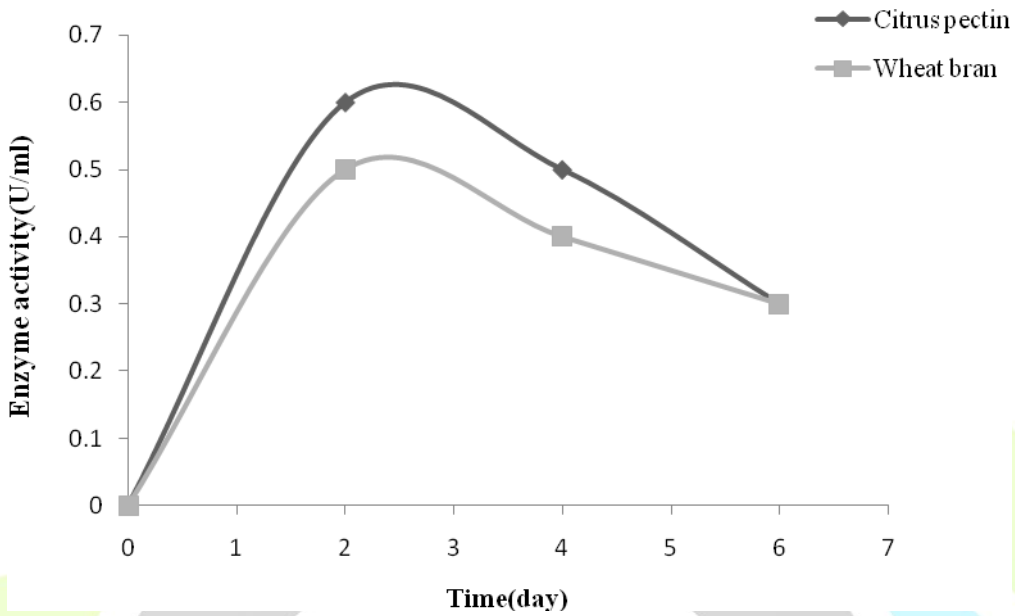

Figure 2: Daily production of Exopectinase using citrus pectin and wheat bran substrates

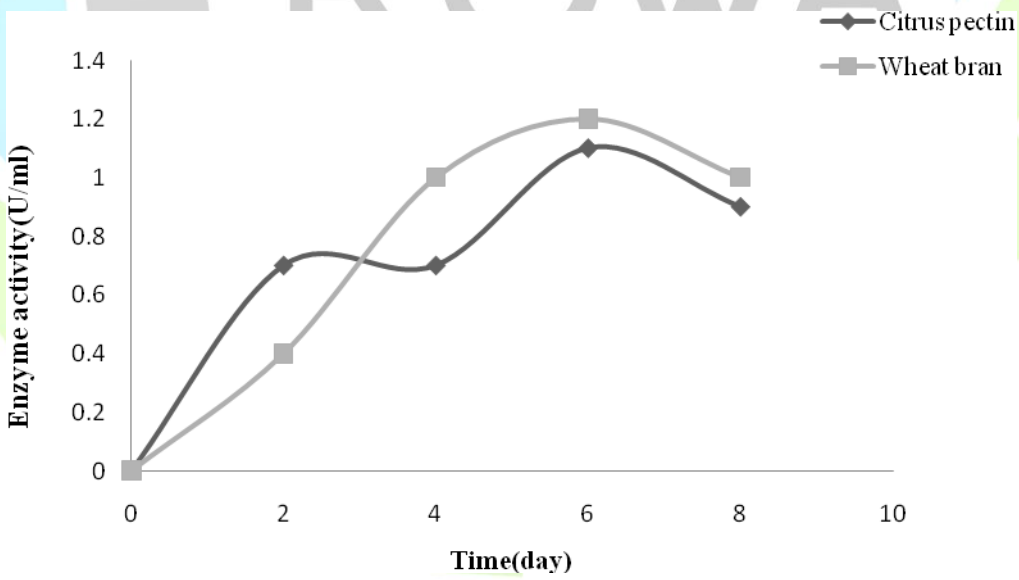

Figure 3: : Daily production of Endopectinase using citrus pectin and wheat bran substrates

DISCUSSION

In the present study we have used the industrial fungus *Aspergillus* terreus PTCC 5013 for pectinolytic enzymes production in bath culture. Exogalacturonse activity assayed based on the amount of D-galacturonic acid which was released in the culture supernatant per minute in 50˚C .

In the most previous studies, the absorbance of reducing sugar in the presence of dinitrosalicilic acid used for exogalacturonase activity assay (Blandino, 2002; Aminzadeh, 2007; Gomes, 2009).

For endogalacturonase activity assay, most of the investigations were based on viscometrical methods (jayani, 2005).

In the present study, the highest exopectinase production achieved in the sixth day after inoculation. It was 0.6 $U.ml^{-1}$ in citrus pectin and 0.5 $U.ml^{-1}$ in wheat bran in bath culture. The highest endogalacturonase production show in the second day. It was 1.1 $U.ml^{-1}$ in citrus pectin and 1.2 $U.ml^{-1}$ in wheat bran. Gomes (2009) reported that the polygalacruronase activity can be increased up to 3.0 to 4.1 $U.ml^{-1}$ by adding substrates such as orange bagasse in batch cultures, so we can elevate the enzyme production by the optimization of culture condition.

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