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RESEARCH ARTICLE

Purification and Properties of Polygalacturonase from a Novel Strain *Aspergillus foetidus* MTCC 10367

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Abstract

The Polygalacturonase (exo PGs) from a newly isolated fungal strain *Aspergillus foetidus* MTCC10367 capable of utilizing citrus pectin as substrate was separated and purified to homogeneity by SEPHADEX G-100 gel filtration chromatography with a Purification fold of 119.59 and enzyme yield 71.55 % was achieved. The enzyme had an approximate molecular weight of 53kDa. Polygalacturonase activity for purified enzyme was found to be 40.1 U/ml, protein concentration was 0.5 mg/ml and specific activity was determined as 80.2 U/mg. The optimum temperature and pH were 60°C and 5.5 respectively. The K_m and V_{max} of the enzyme were 8.33 mM of Polygalacturonic acid and 166.66 U /min/mg respectively.

Introduction

Polygalacturonase (PGs) are the pectinolytic enzymes that catalyze the hydrolytic cleavage of pectin chain with introduction of water across the oxygen bridge (Pedrolli and Carmona 2010). The commercial sources of pectinase include a wide variety of bacteria (such as *B. polymyxa*, *Erwinia spp*, *E. carotovora*, *Ps. syringae*), some yeasts like *Kl. fragilis* and *Kl. Marxianus.(*Amid Mehrnoush 2011.) and filamentous fungi such as *Aspergillus* niger (Maciel *et al*., 2011) , *Aspergillus fumigatus* (Phutella *et al*., 2005), *Aspergillus awamori* (Baladhandayutham and Thangavelu 2011), *Aspergillus sojae* (Tari *et al*., 2008), *Aspergillus clavatus*, *Fusarium sp* (Al-Najada *et al*., 2012) and *Penicillum chrysogenum* (Tariq and Reyaz 2012). Production of Polygalacturonase is mainly done by submerged fermentation (SmF) using *Aspergillus sp.* since they are generally regarded as safe (GRAS) for food industry (Evrim Taşkın, *et al.,* 2008). Purification and biochemical characteristics of enzymes are required for understanding their structure, mechanism and thermostability (Damásio, *et al*., 2009). Generally pectinases from various microorganisms are purified to homogeneity with a combination of ion exchange and gel filtration chromatograph as main procedures (Gummadi and Panda, 2003) while few purification procedures are carried out using affinity adsorption chromatography (Zhang Chun-hui 2009). Fungal PGs are monomeric proteins with a carbohydrate content of 5–81% and molecular masses in a range 13 to 82 kDa (Gomes, *et al*., 2009).

MATERIALS AND METHODS

Microorganism and Enzyme Production

Aspergillus foetidus MTCC10367 was isolated from industrial fruit waste in and around Galla food processing industry, Chittoor District, Andhra Pradesh, for Polygalacturonase production and maintained as stock culture on Potato Dextrose Agar at 4°C. The fermentation medium in a 250 mL Erlenmeyer flask contained: (%w/v) (1% citrus pectin; 0.001 $FeSO_4$ $7H_2O$; 0.05 K_2HPO_4; 0.05 $MgSO_4.7H_2O$; 0.05 KCl; 0.2 $NaNO_3$) was sterilized at 121°C for 15 min, then cooled and pH adjusted to 5.0. The medium was inoculated with a volume of 2mL conidial suspension (1.13×10^7 cfu/mL) and cultured at 28 ± 2°C on a rotary shaker 150 rpm for 5 days. After incubation, the biomass was separated by filtration and culture filtrate was used as source of crude enzyme and stored at 4°C (Sebastian et al., 1996).

Precipitation and dialysis of enzyme

The crude enzyme was subjected to 20-80 % Ammonium Sulphate precipitation (Olutiola and Akintunde, 1979), saturated overnight at 4°C, then centrifuged at 10,000 ×g for 30 minutes (Coelho *et al.,* 1995) and thereby dialyzed using dialysis bag (Himedia, Mumbai).

Gel filtration chromatography

The dialyzed enzyme fraction was applied to SEPHADEX G-100 column (Sigma , USA) The column (2.5 x 50 cm) was equilibrated with Sodium Acetate buffer (0.1M, pH 5.0) and eluted at a flow rate of 1 mL / min with the same buffer. The active fractions were collected and protein content was spectrophotometrically measured at 280 nm (Coelho *et al.,* 1995).

Assay of PG activity

Polygalacturonase activity was measured by determining the amount of reducing groups released according to the method described by Nelson (Nelson, 1944) and modified by Somogyi (Somogyi, 1952).One unit of Polygalacturonase has been defined as the amount of enzyme that releases one µ mole of galacturonic acid /mL/minute under standard assay conditions. The protein concentration was determined using BSA as standard (Himedia) by Lowry's method (Lowry *et al.,* 1951).

SDS–Polyacrylamide Gel Electrophoresis (PAGE)

SDS-PAGE was performed to check the homogeneity of the enzyme on a 12% gel (Laemmli, 1970) and molecular weight determined by standard protein marker (MEDOX-PRO Medox Biotech, Chennai). The protein bands on gel were visualized by staining it with Coomassie brilliant blue G- 250.

Characteristics of purified Polygalacturonase

Effect of Substrate Concentration

The effect of substrate (Polygalacturonic acid) concentration on the activity of enzyme was determined by using different concentrations of Polygalacturonic acid (1-5mM) and the enzyme activity was estimated. The K_m value of the enzyme was also calculated from the data using Lineweaver-Burk Plot.

Characterization on Enzyme Activity and Stability

The effect of pH and temperature on enzyme activity was assayed by varying the pH from 3.0 to 9.0 and temperature from 30 - 80°C. For thermal stability the enzyme was incubated at the temperature conditions 4-75°C for 4 hours. The enzyme activity was determined in every 30 minutes. The stability was assayed by incubating the enzyme at pH conditions 3.0 to 8.0 at room temperature and enzyme activity was determined in every 30 minutes.

RESULTS

The crude Polygalacturonase produced by *Aspergillus foetidus* MTCC 10367 was subjected to 80% Ammonium Sulphate fractionation in the first step followed by gel filtration chromatography on a SEPHADEX G-100 column. Polygalacturonase activity for purified enzyme was found to be 40.1 U/ml, protein concentration was 0.5 mg/ml and specific activity was determined as 80.2 U/mg. A purification fold of 119.59 and % recovery of 71.55 was achieved (Table 1). SDS-PAGE electrophoregram of the purified enzyme revealed a single band with approximate molecular weight of 53kDa (Figure 1). The optimum pH and temperature of the Polygalacturonase were 5.5 and 60°C respectively (Figures 2 and 3). The enzyme was stable up to 65°C for 30 minutes and it was stable up to 30 days at 4°C. The K_m and V_{max} of the enzyme were 8.33 mM of Polygalacturonic acid and 166.66 U /min/mg respectively.

Table1: Purification data of Polygalacturonase from *Aspergillus foetidus* MTCC10367

Purification Steps	Volume (mL)	Total Activity (U)	Total protein (mg)	Specific activity (U/mg)	% recovery	Purification fold
Crude enzyme	100	84	126	0.67	100	1
Ammonium Sulphate fractionation	10	65	6.8	9.56	77.38	14.27
Sephadex G-100	1.5	60.1	0.75	80.13	71.55	119.59

Figure 1: Analysis of Polygalacturonase purification by SDS-PAGE

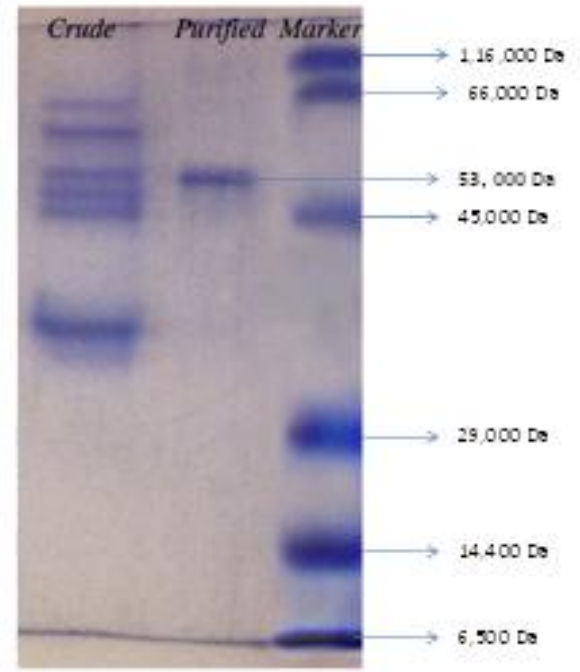


Figure 2: Effect of pH change on the activity of Polygalacturonase

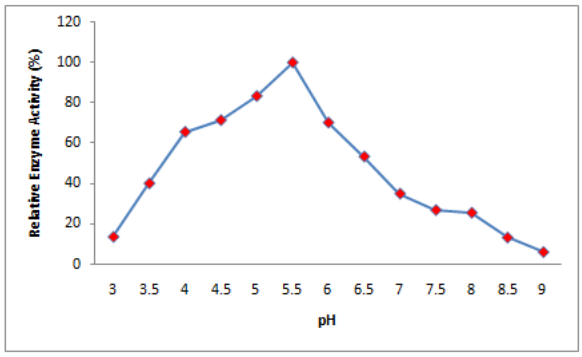


Figure 3: Effect of Temperature change on the activity of Polygalacturonase

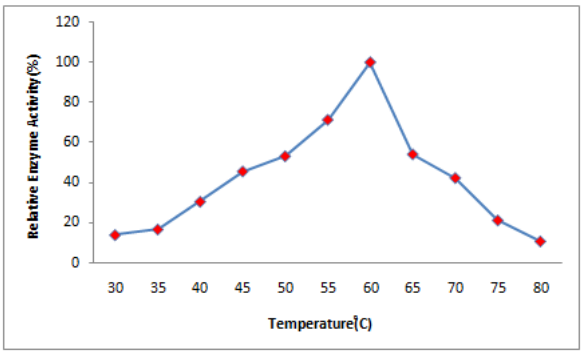


Figure 4: Effect of substrate concentration

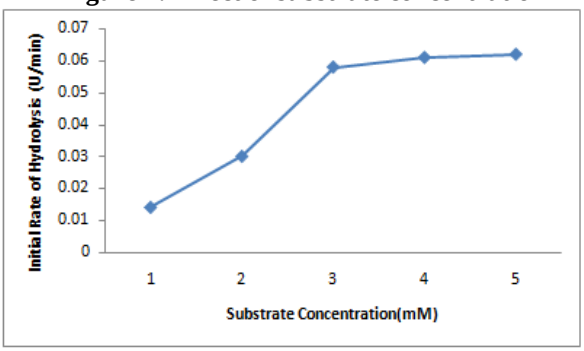
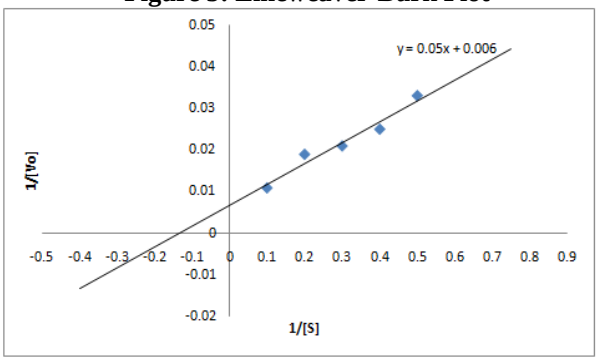


Figure 5: Lineweaver-Burk Plot



DISCUSSION

The fungal Polygalacturonase from *Aspergillus foetidus* MTCC10367 was purified to homogeneity by SEPHADEX G-100 gel filtration chromatography. Studies have shown that Polygalacturonase can be Precipitated between 0 - 90% of Ammonium Sulphate depending on the source of the enzyme (Buga *et al.,* 2010). The majority of purified fungal Polygalacturonase have molecular weight in the range from 25 kDa to 82 kDa (Mohamed *et al.,* 2009). The purified Polygalacturonase from *T. aurantiacus* reported an optimum pH as 5.5 (Martins *et al.,* 2007) whereas for *Thermomyces lanuginosus* an optimum pH 5.5 and optimum temperature of 60°C was reported (Kumar and Palanivelu, 1999). Purified PG from *Aspergillus giganteus* showed maximal activity between 55°C and 60°C (Pedrolli and Carmona, 2010).

The Polygalacturonase obtained from *Aspergillus foetidus* MTCC10367 was precipitated at 80% Ammonium Sulphate fractionation, has approximate molecular weight of 53kDa, has an optimum pH 5.5 and temperature 60°C. These results are comparable with various studies conducted by different researchers using various Polygalacturonase producing fungal species. The study indicates that Polygalacturonase from this particular strain can find various industrial applications owing to its unique properties.

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