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PECTINASE PRODUCTION FROM *ASPERGILLUS NIGER* IBT-7 USING SOLID STATE FERMENTATION

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Abstract

Different fungal strains were isolated from the local soil, fruits and vegetables on the basis of pectin hydrolysis. All the isolated strains were identified through microscopic studies and screened for pectinase production using solid state fermentation. The fungal strain identified as *Aspergillus niger* IBT-7 showed the highest pectinase production. The selected strain was further subjected to optimization through different physical and nutritional parameters to enhance the production of pectinase. Amongst seven different media tested M1 containing rice bran, moistened with Czapek's nutrient medium showed the highest pectinase production. During optimization maximum pectinase production was achieved after 72 hrs of incubation at 30 ml of moisture content, pH 5.0 and 30°C. Xylose (1.5%) and yeast extract (1%) proved to be best supplemented carbon and nitrogen sources, respectively which gave the highest pectinase production (39.1 U/ml/min).

Introduction

Pectinase (EC.3.1.1.11) is a group of enzymes that is involved in the breakdown of pectin. It also breaks glycosidic linkages by splitting polygalacturonic acid into monogalacturonic acid (Sethi *et al.* 2016). It is a type of polysaccharide made of esterified D-galacturonic acid found in α-1-4 chains of middle lamella and primary cell wall of vegetables and fruits. When the ripening process takes place pectinase enzyme breaks down pectin, fruit becomes softer because the cells get separated from each other due to breakage of the middle lamella (Anisa *et al.* 2013). Global food enzymes sales of pectinases account for 25% (Sethi *et al.* 2016). Pectinase has a wide range of applications and is a very important enzyme in food industry such as in fruit juice clarification, in the processing of fruits and vegetables. It is also used in the beverage industry, oil extraction, fermentation of tea and coffee. Other areas of utilization involve the, retting of vegetable fibers, pulp and paper industry, waste management, degumming of plant based fibers and removal of haze from wines (Schwan and Wheals 2004).

Microorganisms and plants are the two leading sources of pectinase. However, pectinase produced from plants does not meet the industrial requirements. Therefore, microorganism could be a preferred source of pectinase because of the high productivity rate, cost effective and short life span. The 50% of total pectinases are obtained from fungi, 35% from the bacteria and 15% from plant. However, priority is given to the fungi because fungi are capable of colonizing the substrate by apical growth penetration and grow easily in the environment having low moisture content. This is an ecological advantage over bacteria which are capable of multiplying on the surface of substrate and cannot easily grow in a low moisture environment (Pereyra *et al.* 1996).The commonly used fungi for the production of pectinases are species of *Aspergillus, Penicillum* and *Rhizopus* (Ibarra *et al.* 2017). Pectinase can be produced by two types of fermen-

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tation i.e. solid state and submerged fermentation. Solid state fermentation has a number of advantages over submerged fermentation. Yield of pectinase is maximum because the growth of organisms is very high. The cultural conditions of fungi are similar to the natural habitat of fungi in solid state fermentation. In addition to high yield there is increased production of biomass, less catabolic repression, low water requirement (Garlapati *et al.* 2015).

Due to increasing industrial demand, the need for pectinase production is increasing periodically. Maximum yield of pectinase can be achieved by using agricultural wastes. The use of wastes product is economically viable, furthermore the production can also be increased after optimization of fermentation conditions and different cultural parameters (Bibi *et al.* 2016).

Materials and Methods

Different samples of soil, fruits and vegetables were collected from agricultural fields and vegetable markets of Lahore and Gujranwala. Pectinase producing fungi were isolated from these samples by the method of serial dilutions using the pectinase screening agar medium (PSAM) plates (Saha *et al.* 2014). Only those fungal isolates were selected which have shown larger pectin hydrolyzing zone. All the selected fungal strains were identified on the basis of micro- and macroscopic characteristics.

Solid state fermentation was carried out using sterilized medium like 10 g of solid substrate moistened with 10 ml of mineral salt medium in 250 of ml conical flasks One ml of inoculum was added in all flasks and incubated at 30°C for 3 days. The inoculum was prepared by adding 10 ml of 0.9% saline water in 4 day-old fungal culture and mixing thoroughly. After a fixed incubation period 100 ml of 0.1 M sodium acetate buffer (pH 6) was added in fermented bran and placed in shaking incubator for one hour. The contents of flask were filtered and filtrate was used for the assessment of pectinase.

Table 1. Different media used for the production of pectinase.

Media	Components (g/l)
M_1	10 g of rice bran, 10 ml of Czapek's nutrient medium containing (g/100 ml): 30 $NaNO_3$; 5 KCl; 5 $MgSO_4$.7H20 and 0.1 $FeSO_4.7H_2O$ (Palaniyappan *et al.* 2009)
M_2	10 g of rice bran, 10 ml of the mineral salt solution containing (g/l): 6.0 $(NH_4)_2SO_4$; 6.0 K_2HPO_4, 6 KH_2PO_4 and 0.1 g $MgSO_4.7H_2O$ (Kunte and Shastri 1980)
M_3	10 g of banana peel, 10 ml of nutrient medium solution containing (g/l): 1.4 NH_2SO_4; 6 K_2HPO_4; 2 KH_2PO_4 and 0.1 $MgSO_4.7H_2O$ (Amande and Adebayo-Tayo 2012)
M_4	10 g of orange bagasse; 10 ml of nutrient solution containing (g/l); 1 NH_4NO_3, 1 $NH_4H_2PO_4$ and 1 $MgSO_4.7H_2O$ (Martin *et al.* 2004)
M_5	10 g of sugarcane bagasse, 10 ml of nutrient solution containing (g/l); 1NH_4NO_3; 1 $NH_4H_2PO_4$ and 1 $MgSO_4.7H_2O$ (Martin *et al.* 2004)
M_6	10 g of rice bran, 10 ml of medium containing (g/100 ml); 0.1 glucose, 0.1 yeast extract and 0.1 $CaCl_2$,Citrate phosphate buffer (Hoa and Hung 2013)
M_7	10 g of sugarcane bagasse; 10 ml of Czapek's nutrient medium containing (g/100 ml): 30 $NaNO_3$; 5 KCl ; 5 $MgSO_4.7H_2$0 and 0.1 $FeSO_4.7H_2O$ (Palaniyappan *et al.* 2009)

Pectinase assay was carried out following the method of Okafor *et al*. (2010). The reaction mixture containing 1 ml of filtrate along with 1 ml pectin (1%), prepared in sodium acetate buffer (0.1 M, pH 5.5) was incubated in water bath at 50°C for 30 min. A blank containing distilled

water instead of filtrate was also run parallel. The reducing sugar was determined according to Miller (1959). The absorbance was taken at 540 nm by using spectrophotometer."One unit of pectinase activity was defined as“ the quantity of enzyme which release 1µmole of galacturonic acid per minute under the assay conditions" (Islam *et al.* 2013).Total protein content was estimated by Bradford (1976).

All the data were arranged and statistical analysis (Post Hoc multiple comparison test under one way ANOVA and standard deviation) was performed by using SPSS (version 23.0.) software.

Results and Discussion

The choice of appropriate strain is vital for successful pectinase production. In the present investigation 25 pectinase producing strains were isolated on the basis of qualitative screening. The quantitative screening of all selected isolates for pectinolytic potential was carried out in solid state fermentation (data not shown). The isolate number seven showed maximal pectinase production (5.50 U/ml/min) given the code IBT-7. The IBT-7 was identified as *Apergillus niger* according to Saha *et al.* (2014).

It is very significant to select an appropriate fermentation medium for enzyme production. The seven different media were tested for pectinase production (Fig. 1a). Among all these media M1 produced a maximum concentration of pectinase (9.5 U/ml/min). The reason might be that rice bran contains all essential factors which support the growth of fungi. In addition to this nutrient present in Cepezek media favoured the fungal growth and secretion of enzyme (Baladhandayutham and Thangavelu 2011). Rice bran was selected as a suitable substrate during the evaluation of fermentation media. Different concentration of rice bran (5 - 30 g) was tested. The 30 g of rice bran gave highest pectinase productivity (Fig. 1b). This might be that rice bran was an excellent substrate which provides anchorage to fungus as well as is rich source of mineral particularly phosphorous, potassium, calcium (Chutmanop *et al.* 2008) Any increase or decrease in this concentration reduced the pectinase activity. At low concentration of rice bran less amount of nutrients are available to fungal growth which resulted in less enzyme production (Kaur and Gupta 2017). Moisture content greatly influence the fungal growth and enzyme production. In this study different concentration (10 - 50 ml) of moisture content for enzyme production was investigated. Moisture content at the level of 30 ml gave optimal pectinase activity (Fig. 1c). Any fluctuation in this concentration resulted decrease in the pectinase production. It might be that high level of moisture content shows negative effects on fungal growth, decrease in nutrients solubility of solid substrate, contaminate the media, reduced porosity of medium as well as oxygen transfer. Moisture content below the optimum level affects the fungal metabolism, and suppresses the fungal growth (Acuria-Arguelles *et al.* 1995). .

The fermentation period depends upon the composition of the medium, type of microorganism, and physiological conditions. In the present study different fermentation periods ranging from 0 - 120 hrs was tested to find out the maximum pectinase productivity (Fig. 1d). Pectinase production gradually increased and reached its maximal point after 72 hrs of imitation. Further increase in fermentation period resulted decline in enzyme production. The cause of this decline might be the exhaustion of essential supplements in the production medium and/or accumulation of toxic auxiliary metabolites (Manal *et al*. 2016).

Temperature is also one of the crucial factors that regulate the fermentation process. It had great impact on growth and metabolic activity of fungal strain. Different temperature range 20 - 50°C was evaluated for the production of pectinase (Fig. 1e). Optimum enzyme production was noted at 30°C. The reduction in enzyme production above or below this optimal level could be due

to less growth of microorganism and reduction in moisture content of medium in solid state fermentation (Kaur and Gupta 2017).

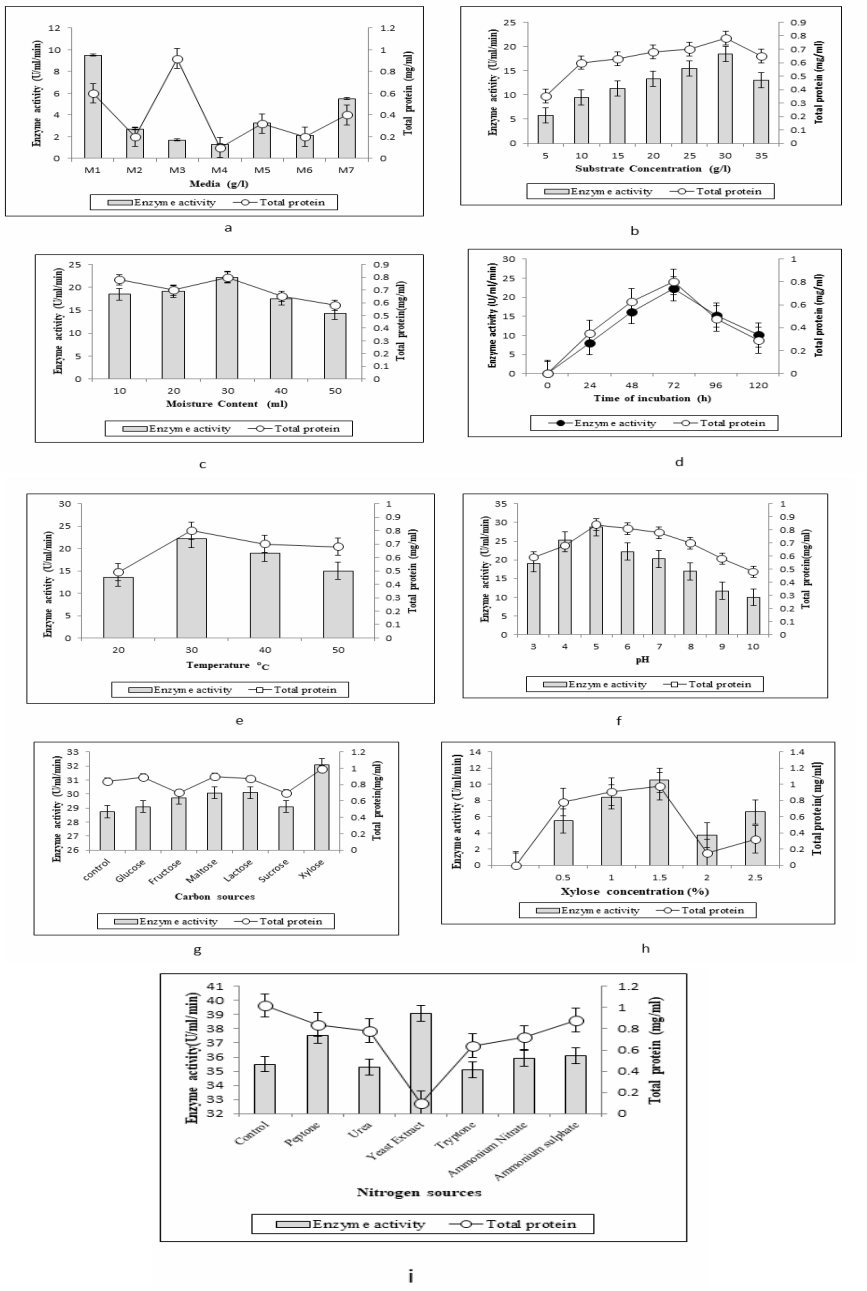

Fig. 1. Influence of different parameters on pectinase production: (a) Media, (b) substrate concentration, (c) moisture content, (d) incubation time, (e) incubation temperature, (f) pH, (g) carbon sources, (h) concentration of xylone and (i) nitrogen sources.

The pH regulates many factors such as the growth of fungal strain, membrane permeability and enzyme stability. The effect of different pH (3 - 10) on the enzyme productivity was tested (Fig. 1f) The result revealed that optimal pectinase productivity was observed at pH 5. Any change in this optimal level resulted decline in enzyme production. Further increase or decrease in pH represented decline in pectinase production because enzyme are very sensitive to any change in the concentration of H^+. pH above the optimum level resulted in less growth of the fungal strain and less enzyme production (Kaur and Gupta 2017). Similar results were also reported by Adeleke *et al.* (2012**)** who reported pH 5 as optimal for pectinase production.

The impact of additional carbon sources such as glucose, sucrose, xylose, fructose, lactose and maltose was also studied (Fig. 1g). Among all the added carbon sources xylose proved to be best for optimal production of pectinase. This might be that xylose acts as an inducer and stimulate the production of enzyme. Different concentrations of xylose (0.5 - 2.5%) for maximal productivity of enzyme was screened (Fig. 1h).The maximal enzyme activity was noted at 1.5%. Above or below the optimal concentration of carbon source caused decrease in the productivity of enzyme (Baladhandayutham and Thangavelu 2011).

The impact of different inorganic and organic nitrogen sources (ammonium sulphate, ammonium nitrate, peptone, urea, yeast extract, tryptone were studied (Fig. 1i). The nitrogen sources improved the fungal growth and stimulated the secretion of enzyme. Among additional nitrogen sources yeast extract gave maximal pectinase production. Because organic nitrogen source such as yeast extract maintains the fungal growth better than that of inorganic nitrogen sources. Fungal strain hydrolyzes the yeast extract easily so, different nutrient components and growth factors which were released assimilated into fungal metabolism that ultimately increases their growth. In addition to this yeast extract provide stimulatory component like vitamin (Qureshi 2012**,** Sethi *et al.* 2016).

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