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PRODUCTION AND PARTIAL PURIFICATION OF PECTINASE BY FUNGAL STRAINS GROWN ON ORANGE PEEL

V. G. Meshram
University Deptt. of Biochemistry,RTM Nagpur University,Nagpur.
vgmesh@gmail.com

Abstract
Abstract The peel of citrus fruits contains a large percentage of pectin which can be a good substrate for pectinolytic microorganisms. These microbes secrete large amount of extracellular enzymes to degrade the cell wall of substrates. The current study was conducted for the production and characterization of pectinase from fungal strains using citrus fruits peel as a substrate. The optimum pectinase production was analyzed from pectinase activity assay. A. niger,A. flavus and A.fumigatus, showed maximum production at 6th day,and A. oryzae,at 4 th day. The optimum temperature for pectinase production by A. niger and A. oryzae was found to be 35Â°C and for A. flavus and A. fumigates was found to be 40Â°C.Pectinase showed optimum pH in the range of pH 4.5-5.0. Pectinase was purified by the addition of 60% of ammonium sulfate precipitation and dialysis and showed various fold increase in pectinase activity.
Keywords: Citrus fruits, pectinase, A. niger, A. flavus, A. oryzae , A.fumigatus.

Introduction:

Citrus fruits are one of the important fruits, produce all over the world. These include orange, Mausami and sweet orange (Dhillon et al., 2004). Nagpur is one of the major producer of orange fruits in India. Pectin is the major component of primary cell wall of all citrus fruits. Pectin is a polysaccharide which have important nutritional and gelleting properties in foods (Mohnen, 2008). Pectinolytic enzymes can be produced in large amount by microorganisms, using citrus peel as a substrate because it contains considerable quantity of pectin. It works as inducer for the synthesis of pectinolytic enzymes by microbial systems (Dhillon et al., 2004). These enzymes have the ability to degrade and chemically modify pectin (Zhang, 2006). Pectinases are commonly employed in juice, textile, paper and pulp industries. These enzymes catalyzed the conversion of complex polysaccharides into simpler molecules like galacturonic acids (Kashyap et al., 2000; Giese et al., 2008). These have wide industrial applications like oil extraction, tea extraction, juice clarification and waste water treatment (Hoondal et al., 2002; Botella et al., 2007; Mohnen, 2008). Microorganisms have various advantages and can be used for enzymes production at higher level. Pectinolytic enzymes have great biotechnological potential and can be employed in many important industrial processes (Tewari et al., 2005; Zhong and Cen, 2005). Aspergillus belongs to ascomycota group of fungi, genus Aspergillus. It is an opportunistically infectious microbe to human being and well adapted to environmental changes (Samson et al., 2001; Baker, 2006). The current study was designed for the optimization and production of pectinase by Aspergillus sp. and then its characterization after partial purification.

Material and Methods:

Substrate preparation Orange peel was used as substrate. It was sliced, air dried and meshed with 40 mm mesh. Fermentative organism and sporulation medium Pure culture of A. niger, A. flavus., A. oryzae and A. fumigates were used. It were maintained on potato dextrose agar (PDA) slants which were inoculated at 30Â° C for 120 hours and stored at 4Â°C for further use(Motwani et al 2012). Numbers of spores were adjusted at 107-108 spores/mL microscope (Kolmer et al., 1951). Screening of fungal isolates for pectinolytic activity The fungal isolates were assayed for pectinase activity using pectin containing agar medium. Culture plates with pectin-containing agarose were inoculated with each isolate and incubated for 3-5 days at 31Â°C. Isolates were replicated 2 to 3 times and tests were performed twice. After incubation, plates were stained with aqueous 0.05% ruthenium red solution for 1h and rinsed with deionised water. Cultures expressing pectinase activity exhibited a clear zone around the margins of the colony. Solid state fermentation All experimental treatments were performed in triplicate flasks containing 5 g substrate moistened with mineral salts solution. Flasks were plugged with cotton and growth medium was autoclaved at standard conditions. 2 mL of inoculum was added under aseptic conditions in autoclaved flasks. These flasks were then placed at 28Â°C temperature

for specific time period. Enzyme harvesting Enzyme was harvested from growth media by sample contact method as described by Krishna and Chandrasekaran (1996). Harvested crude enzyme was stored at 4Â°C before performing enzyme assay. Enzyme characterization Pectinase was characterized for pH and temperature to increase its activity. Tris HCl buffer (50 mM) was used to adjust the pH. of the assay mixture. Enzyme assay was performed at different temperatures for temperature optimization. Partial purification byAmmonium sulphate precipitation Ammonium sulphate is water soluble ionic compound, maintain high ionic strength and precipitate out proteins by salting out. At high ionic strength, salt may remove water of hydration from proteins and reduce solubility, hence proteins were coagulated. Various concentrations of ammonium sulfate were used to obtained maximum precipitation and purification (Motwani et al 2013). Analytical methods Pectinase assay This study focused on the assay of pectinase enzyme for the most active fungal isolate. Pectinase assay was performed following the procedure of Miller, with some modification. Briefly, a reaction mixture composed of 0.2 ml of crude enzyme solution, plus 1.8 ml of 1.0% (w/v) citrus pectin in 50 mM sodium acetate buffer (pH 5.0) was incubated at 37Â°C in a shaker water bath for 30 min. The reaction was terminated by adding 3 ml of DNS reagent. The color was then developed by boiling the mixture for 5 min. Optical densities of samples were measured at 575 nm against a blank containing the reaction mixture minus the crude enzyme. Results were then compared to controls inoculated with an inactive pectinolytic fungal isolate. Results were interpreted in terms of enzyme activity in which one unit of enzyme activity (U) was defined as the mount of enzyme releasing one Î¼mol reducing groups (D â€“ galacturonic acid) per min under these assay conditions. Protein estimation Protein was determined with a Folin-phenol reagent using Bovine Serum Albumin (BSA) as the protein standard(Lowry et al .,1951). Specific activity It is defined as number of units of enzyme activity per mg of protein. Pectinase purification Ammonium sulfate was used for the partial purification of crude pectinase; it precipitate protein by salting out process. Maximum protein was purified at 60% of ammonium sulfate, observed from enzyme activity.

Result and Discussion:

Microbes are the best source to obtain the important enzymes for human needs (Shafique et al., 2009). Enzymes synthesis by microorganisms is affected mainly by substrate, size of substrate particles, surface area of substrate, oxygen utilization, water %, humidity, fermentation temperature, period of incubation and carbon dioxide removal (Jacob and Prema, 2008; Palaniyappan et al., 2009). Pectinases are among the most important industrial enzymes. The biotechnological potential of pectinolytic enzymes from microorganisms has drawn a great deal of attention from various researchers worldwide as likely biological catalysts in a variety of industrial processes.(Kashyap D.R et al.2000) In the current investigation, maximum pectinase activity was observed after 96 h of incubation (Figure 1). With the increase in incubation period, production of enzyme decrease due to accumulation of waste material and unavailability of nutrients. Pectinase has maximum activity in the range of pH 4.5-5.0 of the growth media, indicating that pectinase produced by fungal strains are acidic in nature (Figure 2). Presence of 60% water contents other than inoculums is the most suitable for both, fungal growth as well as pectinase secretion . Similarly, 35Â°C-40Â°C is the most suitable temperature for the growth and production of pectinase by fungal strains (Figure 3). A. niger is a mesophilic fungi, growing well in moderate conditions and temperature.

Table 1: Partial purification of Pectinase by Ammonium sulfate precipitation from *Aspergillus niger*

Source of Precipitation	Enzyme Activity in U/g	Protein Concentration(mg/ml)	Specific Activity in U/mg	Fold of Purification
Crude Enzyme	528	200	2.64	5.65
Ammonium salt Fraction	896	60	14.93	

Table 2: Partial purification of Pectinase by Ammonium sulfate precipitation from *Aspergillus flavus*

Source of Precipitation	Enzyme Activity in U/g	Protein Concentration(mg/ml)	Specific Activity in U/mg	Fold of Purification
Crude Enzyme	480	150	3.2	2.85
Ammonium salt Fraction	640	70	9.14	

Table 3: Partial purification of Pectinase by Ammonium sulfate precipitation forom *Aspergillus oryzae*

Source of Precipitation	Enzyme Activity in U/g	Protein Concentration(mg/ml)	Specific Activity in U/mg	Fold of Purification
Crude Enzyme	496	220	2.25	4
Ammonium salt Fraction	720	80	9	

Table 4: Partial purification of Pectinase by Ammonium sulfate precipitation from *Aspergillus fumigatus*

Source of Precipitation	Enzyme Activity in U/g	Protein Concentration(mg/ml)	Specific Activity in U/mg	Fold of Purification
Crude Enzyme	464	200	2.32	4.46
Ammonium salt Fraction	704	68	10.35	

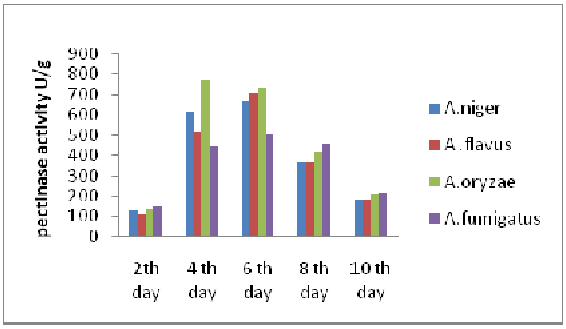

Figure 1 Effect of incubation period on pectinase production.

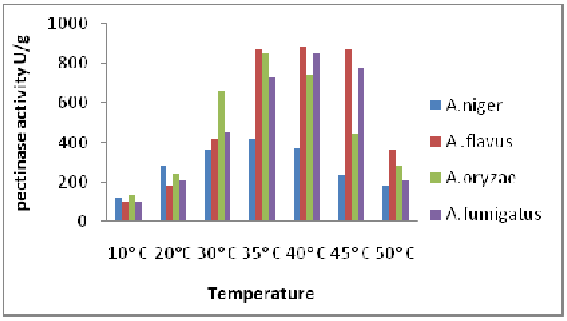

Figure 2 Effect of temperature on pectinase production.

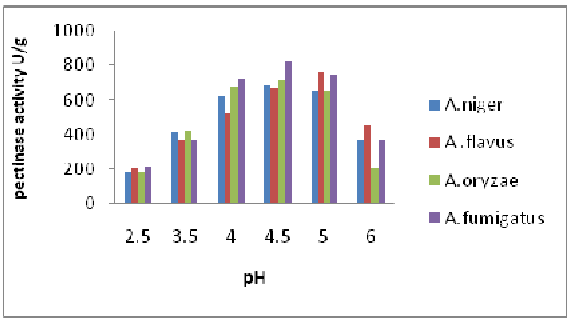

Figure 3 Effect of pH on pectinase production

Conclusion:

From the current study, it can be concluded that Aspergillus sp. can be a good source of pectinase. Supplementation of additional carbon and nitrogen are necessary for good enzymatic yield. In order to achieve further active pectinase further sophisticated purification techniques should be followed.

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