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PECTINASE PRODUCTION FROM *THUJA OCCIDENTALIS* IN SOLID STATE FERMENTATION

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ABSTRACT

The present research work is about the production and process optimization of pectinase enzyme using substrate *Thuja occidentalis* leaves by *Aspergillus awamori* in solid state fermentation. Pectinase having varied applications in food industry such as fruit juice extraction, coffee and tea fermentation, textile, paper and pulp industries and in waste-water treatment. Solid-state fermentation is expressed as a process that take place on a non-soluble material that performs both as support and a source of nutrients, with a reduced among of water, under the action of fermenting agent. Optimizing process evaluations like time, temperature, size of inoculum, pH and moisture content were optimized to induce the high yield of pectinase. The increased level of pectinase enzyme production was detected at time 72hrs, temperature 30°C, optimum inoculum level was 50%v/w, pH 5 and 60% v/w moisture content of the substrate were foremost for the maximum production of pectinase in solid-state condition. A remarkable enzyme production was enhanced and recorded when the basal medium was supplemented with carbon (4% glucose) and nitrogen (ammonium sulphate, 0.4%) sources.

KEYWORDS: *Thuja occidentalis* leaves, *Aspergillus awamori*, pectin, Solid-state fermentation.

INTRODUCTION

Pectinases are enzymes that disintegrate pectin which is polysaccharide that available in plant cell walls. Pectinases are pectic enzymes; they are classified into Pectinesterase, Polygalacturonase and Protopectinase. Pectins are high molecular weight acid polysaccharides, primarily made up of α-(1→ 4) linked D-galacturonicacid remainder with a small number of rhamnose remains in the main chain and arabinose, galactose and xylose on its side chain.[1-4] The mechanism employed to break a peptide bond that involves making an aminoacid residue that has cysteine and threonine or a water molecule nucleophilic to such an extent that it can attack the peptide carboxyl group. Pectinase enzymes are mostly employed in the process of degradation of plant materials, including speeding up the extraction of fruit juice from fruit, like apples and sapota etc. Pectinases are also having abundant industrial applications,[5] food industry, biotechnological industry, coffee and tea fermentation, oil extraction, enhancement of chromaticity and stability of red wines, textile, paper and pulp industries and in waste-water treatment. Solid-state fermentation is defined as a process that take place on a non-soluble material that performs both as support and a source of nutrients, with a reduced among of water, under the action of fermenting agent.[6] Pectinase production has been reported from bacteria including actinomycetes,[7-9] yeast[10,11] and fungi.[12-13] Keep inview of the importance of pectinase enzyme and the advantage of solid state fermentation, the present investigation proposed to study the production and parametric optimization of pectinase enzyme from cheaply and abundantly available raw material.

MATERIAL AND METHODS

Substrate: *Thuja occidentalis* leaves was collected from college garden, Sathupally and dried naturally under sunlight, powdered, packed in a closed container and stored until further use.

Microorganism: *Aspergillus awamori* was used for the production of Pectinase enzyme using *Thuja occidentalis* leaves as substrate. Potato dextrose agar medium was prepared for the maintenance and sub culturing of the microorganism.

Preparation of Inoculum: Streaking is done on pure potato dextrose agar slants from the old culture of *Aspergillus awamori* and streaked agar slants are incubated at 30^0C for 3 days.

Development of Inoculum: 10ml of sterile distilled water were added to 3 days old streaked agar slant, from that 1ml of suspension (approximately 10^5-10^6

spores/ml) was used as the inoculum to each conical flask contain culture medium.

Fermentation: Solid state fermentation was performed in 250-mL conical flask by measuring 100mL of medium contain (in g/L): $(NH_4)_2SO_4$ 0.1, $MgSO_4 \cdot 7H_2O$ 0.5, KH_2PO_4 0.5 and $FeSO_4 \cdot 7H_2O$ 0.005. To carry out the experiment, the initial moisture content of the substrate was adjusted to 50% v/w. The pH adjusted to 5.0 and inoculum level of the medium was 1ml. The flasks were incubated at 30°C. After every 24hrs enzyme assay was carried out and values are recorded.

Enzyme assay: Polygalacturonase activity was detected by measuring the liberated reducing groups from substrate using a 3.5 dinitro salicylic acid (DNS) reagent assay. To the reaction mixture 0.8ml of 1% pectin, 67% methoxylated Braspectina in 0.2M acetate buffer, 0.2ml of crude enzyme solution were added and incubated at 50^0C for 10min. After 10min 1ml of DNS reagent was placed in test tubes and the test tubes are shaken well to mix all the components and to that mixed solution 8ml of distilled water was administrated to avoid the turbidity. The absorbance was detected at 540nm using spectrophotometer. The enzyme and substrate blanks are run simultaneously. One enzyme unit of endopolygalacturonase (EC 3.2.1.15) is the number of micromoles of reducing sugars valuate in terms of monogalactauronic acid, produced as a result of the action of 1ml of enzyme extract in one minute at 30^0C temperature.

RESULTS AND DISCUSSIONS

The production of pectinase is moderated by nutritional, physical and chemical factors. Microbes are used for the production of various enzymes that are helpful for peoples need. The present research focuses on the influence of various physical and chemical parameters for production enzyme. For this different experiments are performed in triplicates and the results noted are the mean values. The nutritional parameters could be effectually keeping in track, for the maximum enzyme production of end product by keeping physicochemical parameters as constant. To identify the effect of time on enzyme production, the medium was incubated at different time intervals, after every 24hrs enzyme assay was carried out and values are recorded. The maximum Pectinase enzyme activity was noted at 72hrs. After 72hrs, it was decreased due to depletion of nutrient materials. Pectinase production at different time intervals is shown in the fig.1.

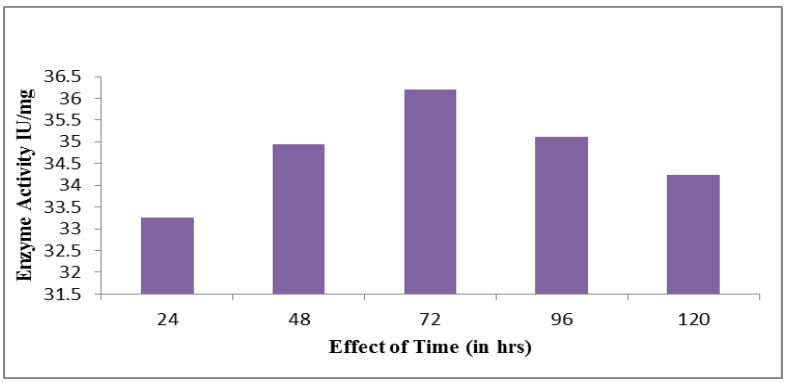

Fig. 1: Effect of time on enzyme production.

The temperature of the substrate is very crucial in solid state fermentation as it ultimately affects the growth of the microbes, spore formation and germination. The maximum amount of Pectinase was noted at 30^0c temperature Fig.2. By increase in temperature, decreased pectinase enzyme activity was observed due to heat inactivation of the enzyme.

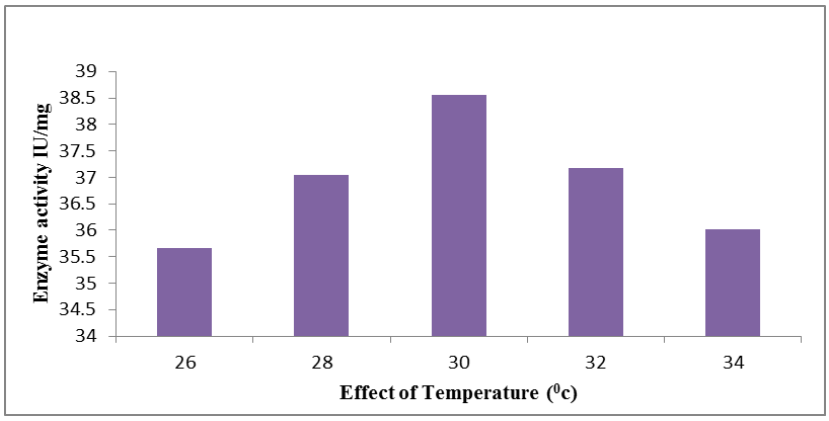

Fig. 2: Effect of temperature on enzyme production.

To determine the effect of pH, the bacterial nutrient medium was adjusted with different pH ranges 3, 4, 5, 6, and 7.0. The maximum production of Pectinase was recorded at pH 5 fig.3.

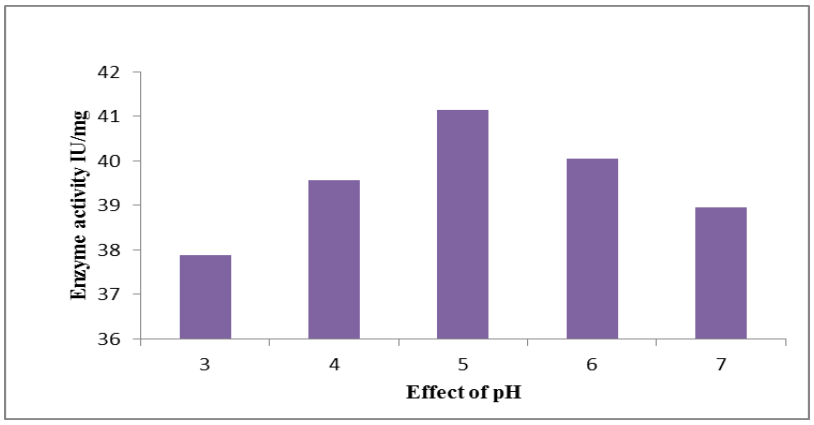

Fig. 3: Effect of pH on enzyme production.

Different inoculum levels were prepared for the production of enzyme 30%, 40%, 50%, 60%, 70%, 80%, 90%v/w. The maximum enzyme production was observed at 50% v/w of inoculum fig.4.

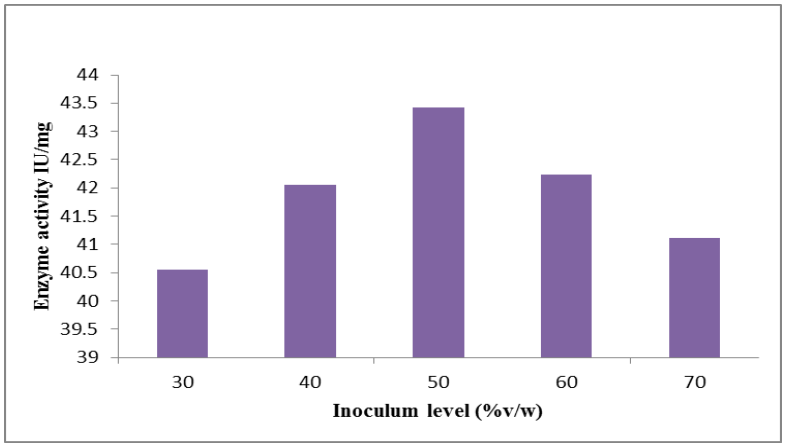

Fig. 4: Effect of inoculum level on enzyme production.

Moisture content of the substrate is predominant for the production of enzymes in solid state fermentation. High moisture content results in decreased substrate porosity, which in turn prevents oxygen penetration, it may cause bacterial contamination. To ascertain the effect of moisture content on enzyme production, Different moisture contents 40%, 50%, 60%, 70%, 80%, 90%, and 100% v/w were prepared and added in each conical flask. The maximum activity was observed at 60% v/w of the moisture content fig.5.

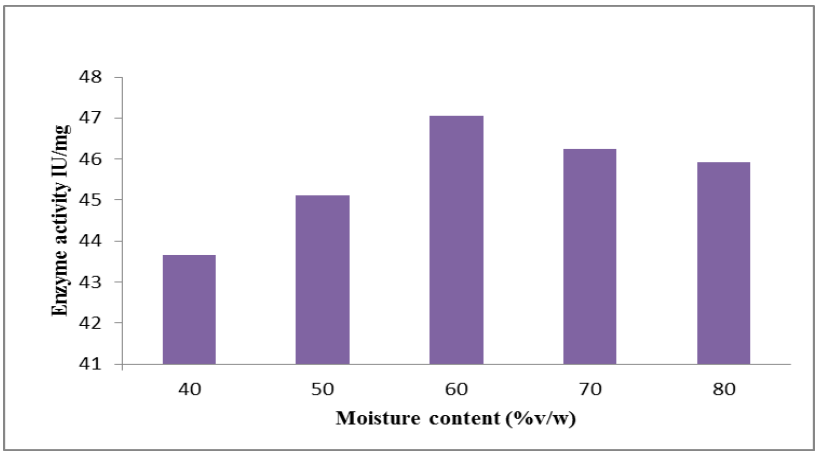

Fig. 5: Effect of Moisture content on enzyme production.

Five different carbon components were screened for the production of Pectinase enzyme which includes sucrose, maltose, glucose, fructose, and lactose. These are enriched with %w/w. The results indicate that glucose supplementation gave marginally improved enzyme than other supplementations. Production medium was prepared with different concentrations of glucose like 1, 2, 3, 4, 5 and 6 %w/w. The result shows maximum enzyme production was observed at 4% w/w of glucose concentration fig.6.

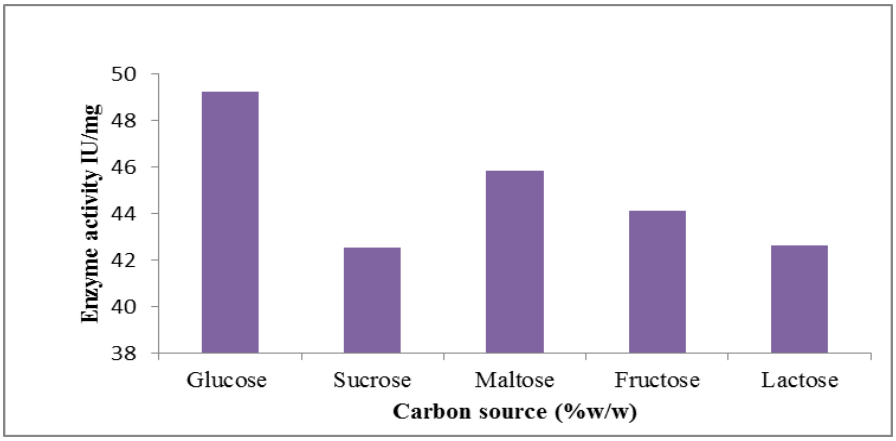

Fig. 6: Effect of carbon source on enzyme production.

To determine the effect of nitrogen on the enzyme production, the production medium was prepared with different concentrations of ammonium sulphate like 0.1%, 0.2%, 0.3%, 0.4% and 0.5%w/w were dispersed in 250ml conical flasks. The result indicates that maximum enzyme production was observed at 0.4% w/w concentration fig.7.

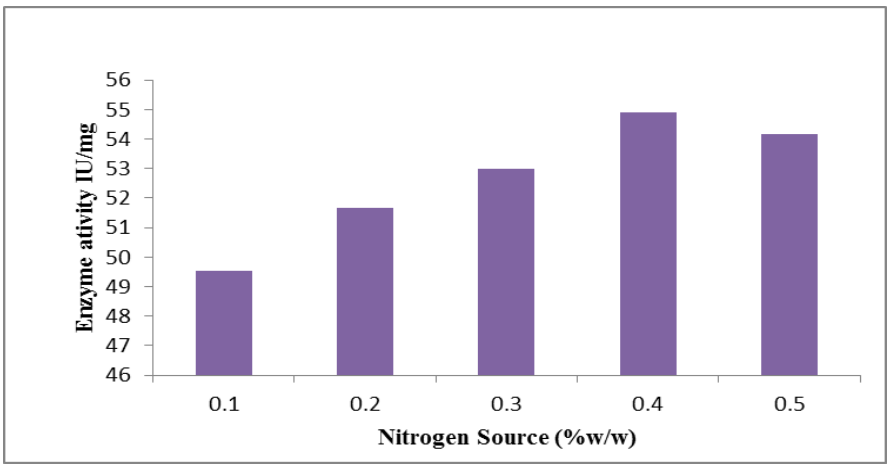

Fig. 7: Effect of nitrogen source on enzyme production.

CONCLUSION

In conclusion, the authors reported production and optimization of pectinase enzyme from cheaply and abundantly available raw materials. Production of pectinase at optimum time, temperature, pH and moisture content enhance the enzyme yield. A remarkable enzyme production was enhanced and noticed when the basal medium was supplemented with carbon (glucose) and nitrogen (ammonium sulphate) sources.

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Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

1. Lang H, Do-Renberg H. Perspectives in the biological function and the technological application of polygalacturonases. Appl. Microbiol. Biotechnol, 2000; 53: 366–75.
2. Deul H, Stutz E. Pectic substances and pectic enzymes. Adv. Enzymol, 1958; 20: 341–82.
3. Kapoor M, Beg QK, Bhushan B, Dadhich KS, Hoondal GS. Production and partial purification and characterization of a thermoalkalistable polygalacturonase from Bacillus sp. MG-cp-2, Process Biochem, 2000; 36: 467–73.

4. Singh SA, Plattner H, Diekmann H. Exopolygalacturonate lyase from a Thermophilic Bacillus sp, Enzyme Microb. Technol. 1999; 25: 420–25.
5. Jayani RS, Saxena S, Gupta R. Microbial pectinolytic enzymes: a review. Process Biochem, 2005; 40: 2931–44.
6. Couto SR, Sanroman MA. Application of solid-state fermentation to food industry – a review. J. Food Eng. 2006; 76: 291-302.
7. Cao J, Zheng L, Chen S. Screening of pectinase producer from alkalophilic bacteria and study on its potential application in degumming of ramie. Enzyme Microb. Technol, 1992; 14: 1013– 16.
8. Bruhlmann F, Kim KS, Zimmerman W, Fiechter A. Pectinolytic enzymes from actinomycetes for the degumming of ramie bast fibers. Appl. Environ. Microbiol, 1994; 60: 2107–112.
9. Beg QK, Bhushan B, Kapoor M, Hoondal GS. Effect of amino acids on production of xylanase and pectinase from Streptomyces sp. QG-11- 3. World J. Microbiol. Biotechnol, 2000; 16: 211–13.
10. Reid I, Ricard M. Pectinase in paper making: solving retention problems in mechanical pulps bleached with hydrogen peroxide. Enzyme Microb. Technol, 2000; 26: 115–23.
11. Blanco P, Sieiro C, Villa TG. Production of pectic enzymes in yeast. FEMS Microbiol. Lett, 1999; 175: 1–9.
12. Huang LK, Mahoney RR. Purification and characterization of an endo-polygalacturonase from Verticillum albo-atrum. J. Appl. Microbiol. 1999; 86: 145–46.
13. Gummadi SN, Panda T. Purification and biochemical properties of microbial pectinases: a review. Process Biochem, 2003; 38: 987–96.