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Effect of temperature on commercially applicable Pectinase enzyme derived from Strain improved Aspergillus Niger using Agro-industrial waste by solid state fermentation process

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Abstract
Pectinase is a general term for enzymes that break down pectin. These are widely distributed in nature and are produced by bacteria, yeast, fungi and plants. Chemically pectinase is poly (1, 4 a-D) galctouronide glycano hydrolase, poly (1, 4-a-d galacturonide lyase, and pectin pectyl hydrolase. Based on it's mode of action and substrate preference these enzyme are classified into Protopectinases, which solubilize protopectin, forming soluble pectin, Esterase [pectin methyl esterase and pectin acetyl esterase] which eliminate methoxy and acetyl residues from pectin giving rise to polygalcturonic acid, and Depolyrnerases, which break the glycoside (1-4) bonds b/w galacturonis residue via, hydrolysis (Polygalacturonases) and trans elimination (Pectin lyases and pectate lyase). The enzymatic activity of pectinase differs with temperature and like all other enzymes it also has a range of temperature for maximal activity. The present study is focused on the effect of temperature on commercially applicable Pectinase enzyme derived from Strain improved *Aspergillus Niger* using Agro-industrial waste by solid state fermentation process. Though the derived pectinase showed activity till 90^0c the maximal activity was at 60^0c.Pectinase enzyme has commercial values in different industries such as Food industry, Textile industry, Paper industry, Poultry industry etc.
***Keywords*:** Pectinolytic enzyme, Microbes in pectinase, Optimal Temperature, Fermentation condition and Industrial Application.

Introduction
Pectin is a structural polysaccharide found in primary cell wall and middle lamella of fruits and vegetables. The predominant structure of pectin is homopolymeric, made of a partially methylated poly-a-(1, 4)-galacturonic acid. Sections of a-(1, 2)-L-rhamnosyl-a-(1, 4)-D-galacturonosyl containing branch-points with L-arabinose and D-galactose can be incorporated in the main polymeric chain. Pectin may also contain residues of D-glucuronic acid, D-apiose, D-xylose and L-fucose attached to poly-a-(1, 4)-D-galacturonic acid sections (*1, 2*)**. [1]**
Pectic enzymes have two classes namely pectinesterases and pectin de polymerases.Pectin esterase has the ability to de-esterify pectin by the removal of methoxy residues. Pectin depolymerases readily split the main chain and it was further classified as polygalacturonase (PG) and pectinlyases (PL).Thus on the whole pectinases are hydrolytic enzymes, which hydrolyze the pectin molecules and are readily soluble in water.

Fermentation Process.
Fermentation is a technique of biological conversion of complex substrates into simple compounds by various microorganisms such as bacteria and fungi. It typically refers to the conversion of sugar to acids, gases and/or alcohol using yeast or bacteria. It is a form of anaerobic digestion that generates adenosine triphosphate (ATP) by the process of substrate level phosphorylation. Two types of fermentations can be carried out for the pectinase production. They are solid State fermentation and submerged fermentation. Solid- state fermentation is traditionally defined as those processes in which microbial growth and products formation occur on the surfaces of solid substrates in the near absence of free water. In comparison between these two fermentations, solid state

fermentation is much better over submerged state fermentation. This system offers numerous advantages over submerged fermentation system, including; High volumetric productivity, relatively higher concentration of the products ,less effluent generation, requirement of simple fermentation equipments, easy product separation, no foam generation and elimination of the need for rigorous control of many Parameters during fermentation**.H.kh.Q at al [4]**

Substrate Used for Pectinase production.

The various agro industrial wastes, such as wheat bran, rice husk, corn straw, corncob, fruit peel, orange and sugarcane baggase, apple pomace, pineapple fiber etc have been utilized for enzyme and other valuable commercial products as they are rich in substance like cellulose, starch, lignin, xylan and pectin and thus are utilized by micro organism as the carbon and energy sources.

Utilization of such kind of agro industrial waste in the production of industrial enzymes can be considered as one of the solid waste disposal process**. Sabika at al [5]**

Strain improvement Strain improvement is defined as deliberate efforts or interventions done to improve the microbe's productivity or characteristics with a view to enhance its output. .Besides, strain improvement aims at changing unused co-metabolites, improving the use of carbon and nitrogen sources and improvement of morphology of cells to be better cells in order to separate the cells and its products.

MATERIALS AND METHODS

Microorganism: The fungus used in this study was *Aspergillus niger* ATCC16404 strain which was obtained from Microbiologics, and cultured on Potato dextrose Agar media at 25±2.5 °C for 7days.

Strain Improvement

Optimization of the environment or nutrition and physical parameters can change the enzyme production potential of any microorganism. .We in our study optimized the carbon source for *Aspergillus nigerATCC16404* strain by deliberately adding increasing concentration of pectin in their growth media and studied its effect on their enzyme production capabilities.

Aspergillus niger ATCC16404 strains were grown in to 5 sets of pectin enriched PDA media with pectin concentration as 0.2%, 0.4%, 0.6%, 0.8% and 1% by weight.

The strains cultured on PDA media were incubated at 28±2 °C for 5.0 days.

The resulting 5 strain were again grown on SSF media having agro industrial waste substrate viz: dried orange peel, wheat bran, and sugarcane baggase. It was found that. strains propagated on 1% pectin enriched media had highest pectinase production. These were selected as the most improved strain and thus were the chosen candidates for further enzyme production

The quantitative assay of pectinase produced by various strain was done by pectin agar plate. The assessment of the quantity of pectinase was in accordance to its ability to clear off a proportionate area on agar plate.

Culture media: For enzyme production the three substrates were dried and powdered prior to the usage as it leads to more surface area interaction with the microbe and thus higher yield. The production media consisted of dried150g orange bagasse, 100g wheat bran and 50g sugarcane bagasse in the ratio of 3:2:1. The media had nutrient component containing: 2g $(NH_4)2SO_4$, 0.5gNaCl, 0.3g$CaCl_2$, 2g $(NH_4)2HPO_4$, 0.6g KH_2PO_4, 3g$ZnSO_47H_2O$, 5g$FeSO_47H_2O$ and 0.3g $MgSO_4$.7H20 in the volume of 300 ml.

The whole content except antibiotic were mixed together at 121± 2.0ºC & autoclaved for 30 minutes. After cooling the whole content tetracycline was added Media was incubated at room temperature (Ambient) for 6-7days.

Extraction of culture filtrate

.After incubation 2.0 % sodium benzoate solution one liter (previously pH 4.5 maintained) was added into the incubated media and kept for overnight to destroy the entire fungal spore. After that the whole material was filtered with musculin cloth. It was then centrifuged at 4000 rpm for 15 minutes and filtered through whatman filter paper .

This filtrate was used as crude enzyme for assay of pectinase activity. Extracted solution was taken in a screw cap Erlenmeyer flask. and preserved at 4◦C in refrigerator.

Pectinase activity assay: The activity of pectinase enzyme can be demonstrated by production of juice from fresh untreated juice. Pectin prevents material, including the juice of the fruit, from settling out from the cells. When it is acted on by the enzyme pectinase, the juice can be easily separated .The pectinase enzyme, allows the juice to settle and to be easily separated from the other material.

We Took three Test tubes .The first test tube was labeled as blank and acted as negative control. To each of the test tube we added 10mL fresh untreated juice and 10mL DM water. We Added 1mL pectinase to the second Test tube, and commercially available preparation of Pure pectinase(NOVAZYME) in the third Test tube. Pure pectinase acted as positive control. Kept the test tubes in a water bath at 50°C for 30 minutes

Settling down of pectic material indicated pectinase activity and thus the recovery of clear juice No juice was recovered in the first test tube as it contained no enzyme and acted as negative control thus proving that juice production does not occur in absence of enzyme. Juice production occurred in the third test tube containing pure pectinase demonstrating that enzyme is necessary for juice production. Juice production occurred in the second, test tube indicating the presence of enzyme.

EFFECT OF TEMPERATURE

In order to study the effect of temperature on enzyme 10ml of enzyme was taken in each of the 5 test tubes. These tubes were heated separately in water baths with temperature set at 50^0c,60^0c,70^0c,80^0c and 90^0c respectively. From each of these test tubes 1ml of the enzyme was pipetted and was put on pectin agar plate in drops. These plates were incubated overnight at room temperature. The pectinase activity was assessed by the zone of clearance cleared on the agar plate where the area was in proportion with the level of enzymatic activity.

The maximal enzymatic activity was found at 60^0C which was depicted by the maximal area of clearance on pectin agar plate. It was found that enzymatic activity decreased as the temp rose further.

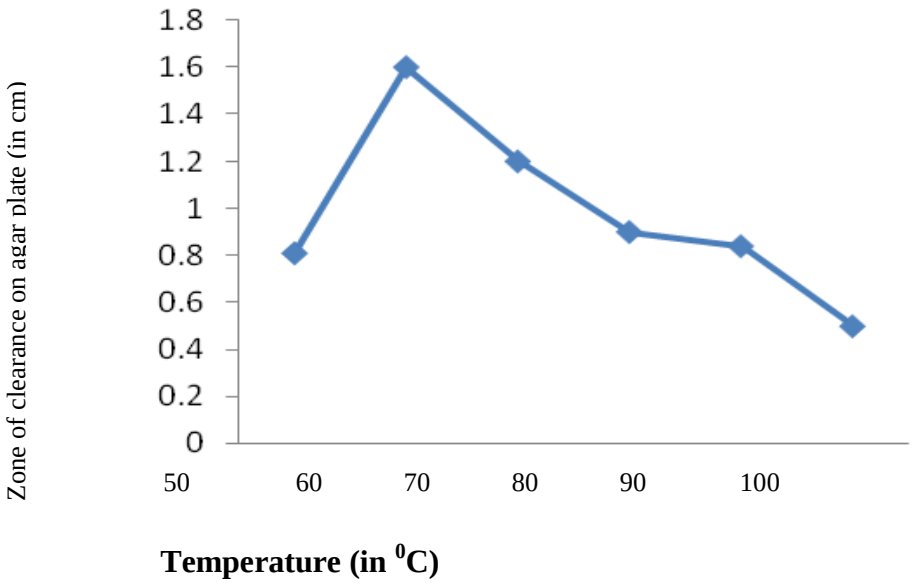

Fig 20 The effect of temperature on pectinase production with temperature ranging from 50ºC-90ºC. The optimum temperature of 60ºC gave the highest enzyme activity Y Axis represents area of zone of clearance on agar gel plate & X axis represents Temperature in ºC.

DISCUSSION

In the present study we used *Aspergillus Niger ATCC16404* strain for the production of industrially applicable pectinase enzyme by solid state fermentation process using agro industrial waste. The cost of production came to be very low as it utilizes only agro waste and the production did not require any stringent conditions as it can be easily produced at room temperature. Strain improvement was

achieved by optimizing the carbon source of the fungus. We in our study optimized the carbon source for *Aspergillus niger* strain by deliberately adding increasing concentration of pectin in their growth media and studied its effect on their enzyme production capabilities. Strain was grown in to 5 sets of pectin enriched media with pectin concentration as 0.2%,0.4%,0.6%,0.8% and 1% by weight. A study by **Ashfaq at al [2]** had also studied the effect of pectin enrichment on Aspergillus niger but only a single case of 5% was used. A wide range of pectin concentrations gave us the more accurate idea about the optimum concentration of pectin for the maximum yield.

In order to study the effect of temperature on enzyme, it was taken in each of the 5 test tubes. These tubes were heated separately in water baths with temperature set at 50^0c,60^0c,70^0c,80^0c and 90^0c respectively

. The aliquots were drawn from each tube and were immediately subjected to pectin agar plate for the enzymatic activity assay, (assessed by the area of clearance) It was observed that the maximum area of clearance and hence the activity was seen at 60^0C. A further increase in temp led to the decrease in enzymatic activity possibly due to heat denaturation of enzymatic protein. Our observation is in contrast to the finding of **Devi at al [3]** who reported optimum temp at 45^0C for Aspergillus species for pectinase production.

The higher optimum temperature of the enzyme prepared can have novel applications in juice and processed food industry as for preservation requires enzymatic activity at high temperature. The new bioactive detergents which enzymatically degrade the biological dirt have a limited range of temperature for their action. This high heat stable pectinase may find its application in a more heat stable bioactive detergent.

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