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Screening Of Pectinolytic Fungi And Optimization Of Process Parameters Using Guava Peel Powder As Substrate Under Solid State Fermentation

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Abstract: *The disposal of waste from fruit processing industries is a major problem. Pectinase is widely used in the fruit industry for clarifying juices. So the waste from fruit industry can be utilized as a substrate for the production of pectinase by identifying new strains which can produce more amount of enzyme as the need of production of high amounts of pectinase is recommended. In this work, pectinases-producing filamentous fungi (P1) was isolated from the soil collected from fruit waste dump, with the aim of finding optimized condition for maximum production of pectinase. In the present study, the production of pectinase using Aspergillus niger NCIM 616 and isolate P1 was carried out under solid-state fermentation using guava waste powder as substrate. Both the fungi showed more or less similar pectinolytic activity. Among the isolates of G3 and P1, the P1 showed maximum pectionlytic activity. Different parameters optimization processes were investigated on SSF namely fermentation time (144 & 120 hr), temperature (30^{0}C), pH (8 & 6), moisture content (160% & 100%), inoculum volume (3ml & 3ml), inoculum age (120 & 144 hr) for Aspergillus niger and P1 isolate respectively.*

Keywords: *Pectinase, Aspergillus niger, P1 isolate, Guava waste, solid state fermentation.*

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I. Introduction

Solid-state fermentation (SSF) is defined as fermentation which involves solids as substrate which has enough moisture to support growth and metabolism of micro-organism [1]. SSF provides opportunities to processes agro industrial wastes and provides a solution for solid waste disposal. SSF has low energy requirements and produce less waste water. Substrates which require either value addition and/ or disposal are used. Pectinase production by SSF using agro industrial residues is feasible [2].

Pectinases hold leading position in commercially produced enzymes and gradually increasing their market. Pectinases has wide range of applications in various industries like fruit and vegetable processing, textile industry, wine industry, processing animal feed, extraction of vegetable oils, tea and coffee processing, bioleaching of Kraft pulp, Recycling of waste paper [3]. Pectinases break down the $\alpha\ 1 \rightarrow 4$ linkage present in the back bone of polygalacturonic acid[4]. Pectinases have been classified based on their mode of action in depolymerization. Hydrolases break down pectic acid or pectin by hydrolysis. Lyases break down pectin by β-elimination. Methyl esterases break down pectin by de-esterification.[5]

Guava (Psidium guajava L.) is a tropical fruit which usually consumed as fresh and it stands in 4th place for its cultivation about an area 0.15 million hectare producing 1.80 million tones of fruit. The ripened guava is easily spoiled when kept at room temperature. So it is processed in various commercial guava products like puree, paste, canned slices in syrup and juice [6, 7]. Guava has almost 83% moisture and is an excellent source of pectin. Guava seeds are rich source of micro nutrients. So the seeds and peel of guava can be used as substrate for production of Pectinase [8].

Aspergillus niger is the widely used microorganism for production of Pectinases as fungi produce large quantities of different enzymes belonging to Pectinases [9]. Presence of pectin in fruit juices causes cloudiness to the juice which is the main problem in producing clear juices. To remove pectin in juice it is treated with Pectinases [10]. Because of their tremendous importance in fruit juice industry the present study focus on selecting new strains of fungal species and comparing them with already existing industrial fungal strain.

II. Materials and Methods

2.1 Microorganism

Pectinase producing fungi is isolated from the soil samples collected from local fruit market waste and was maintained on Potato Dextrose Agar (PDA) slats at 4^0C. Aspergillus Niger NCIM 616 was produced from National Collection of Industrial Microorganisms (PUNE). The culture was maintained on PDA slats at 4^0C. Inoculum is prepared by adding 0.01% tween 80 solution to fully grown slants.

2.2 Isolation of Pectinase Producing fungi

5g of each soil sample was mixed in 100 ml of sterile distill water taken in 250 ml Erlenmeyer flasks and incubated at 28°C at 150 rpm for 24hr. The soil solution is diluted in different proportions from 10^{-1} to 10^{-9} in sterile distill water dilution test tubes by taking 9ml distill water and 1ml soil solution from previous dilution test tube. The soil solution is added to Pectin Agar Media (PAM) by pour plate method.

2.3 Pectin Agar Medium

Pectin agar medium (1000ml) was prepared by weighing pectin 10 g, $(NH_4)_2SO_4$ - 1.4g, K_2HPO_4 -2g, $MgSO_4.7H_2O$ – 0.02%, $FeSO_4.7H_2O$- 0.005g, $MnSO_4.H_2O$- 0.0016g, $ZnSO_4.7H_2O$- 0.0014g, $CaCl_2$- 0.002g, agar – 20g, distilled water 1000 ml taken into flask and autoclaved at 120°C and 15 lbm pressure. The sterilized medium was cooled and Rifampicin was added to inhibit the growth of bacteria. After adding soil solution petriplates are incubated for 7 days at 35°C. After incubation plates are flooded with 0.3% I_2 and 0.6% KI solution [11].

2.4 Substrate

Guava fruit peel and seeds waste collected from fruit pulp factories, dried in sunlight and grinded to powder to use as substrate

2.5 Solid State Fermentation

The SSF has been carried out in 250 ml Erlenmeyer flask by taking 5 grams of Substrate and is moistened with 5ml distilled water at pH 7. The medium was sterilized at 121^0C and 15 lbs. pressure for 20 min. After cooling the media was inoculated with 1ml of spore solution made from 5 day old PDA slants. The fermentation process is carried out at 30^0C for 5 days. After that 25ml of distilled water is added to flasks and kept in orbital shaking at 100 rpm for 1 hr. The mixed solution is filtered using Whatman filter paper and the filtrate was centrifuged at 4^0C at 6000 rpm for 20 min. The supernatant is collected and which is the crude enzyme needed for the enzyme assay.

2.6 Enzyme assays

Pectinase activity was determined by measuring the release of reducing sugar from pectin break down by the enzyme. The mixture containing 0.8 ml 1% pectin (Himedia, India), 0.2ml crude enzyme and 2 ml of sodium acetate buffer. This was kept at 40^0C for 10 min. The reaction is stopped by adding 1ml of DNS reagent and 1ml of NaOH. The tubes were boiled in water bath until the color change observed. Then the solution is diluted to 10ml and absorbance was read at 540nm in UV visible spectrophotometer. The concentration of sugar was determined using galacturonic acid standard curve [12]. One unit of pectinase activity (U) is defined as the amount of enzyme that liberates 1 µM of galacturonic acid per min

2.7 Optimization of process parameters

To improve the enzyme yield in production various parameters were studied and optimized. The effect of incubation time on enzyme production was determined by incubating the inoculated flaks for 3-10 days and activities were estimated for every 24 hr. the effect of temperature was studied by incubating from 20^0, 25^0, 30^0, 35^0, 40^0, 45^0 and 50^0C. Influence of pH was studied by adjusting pH from 2 to 9 by varying one unit. Effect of moisture content studied from 60, 80, 100, 120, 140, 160, 180 and 200% (w/v %). Influence of inoculums age studied from 3rd day to 8th day. The effect of inoculums volume from 1ml to 8ml had been studied.

III. Results And Discussion

3.1 Selecting potential Isolate

Two isolates from the soil samples showed pectinolytic activity. They were named as P1 and G3. Among them P1 showed maximum pectinolytic activity (Fig 4.1). Previous works showed that they also isolated 3 strains of pectinase producing bacteria from agriculture waste soil [13].

3.2 Optimization of process parameters

Optimization studies of different parameters were done for P1 and A. niger and their results were compared. A. niger and P1 showed maximum activity of 71.036 U/ml and 110.92 U/ml for incubation time of 120 hr and 144 hr respectively (Fig 4.2). At the temperature of 30oC both A.niger and P1 showed maximum activity of 74.34 U/ml and 115.64 U/ml respectively (Fig 4.3).

According to Sumi Barman increase in incubation time result in the increase in enzyme activity after the supplied nutrients were consumed then the activity will decrease. Increase in temperature will result in

increase of activity up to certain level after further increase will result decrease in activity. They showed similar results for incubation time and temperature as 65.82 hr and 32.37oC respectively. [14]

By varying pH of the media the maximum enzyme activity of 90.624 U/ml and 133.34 U/ml was observed at pH 8 and pH6 for A. niger and P1 respectively (Fig 4.4). According to R. C. Patil selected isolate has shown maximum activity at pH 9. [15]

By varying initial moisture content results indicated that it had significant effect on Pectinolytic activity. A.niger showed maximum pectionolytic activity at Initial moisture content of 160 % and P1 showed maximum activity at 100% with enzyme activity of **125.08** U/ml and **141.6** U/ml respectively (Fig 4.5). Leeda et al., observed the results of highest pectinase activity obtained at an Initial moisture content of 70% with soy and wheat bran as substrate using Aspergillus niger. [16]

Effect of Inoculum volume was studied and observed the increase in inoculum volume up to 3 ml resulted in the increase of activity. Further increase of inoculum volume resulted in decrement of activity. This may be due to overcrowding of spores. A.niger showed maximum pectionolytic activity at inoculum volume of **3** ml and P1 showed maximum activity at **3** ml with enzyme activity of **153.636** U/ml and **165.2** U/ml respectively (Fig 4.6). Reda Bayoumi et al. showed optimal enzyme activity of B. firmus-I-10104 on Solanum tuberosum (ST) peels was 1 ml [17].

The effect of inoculum age Maximum Pectinolytic activity was obtained with 120 hr old culture for A. niger, 144 hr day for P1 with maximum activity of **176.8** U/ml and **179.36** U/ml respectively (Fig 4.7). Guneet kaur and T satyanarayana reported maximum pectinase production using 4 day-old culture ($6x10^7$ conidiospores) with wheat bran and citrus pectin mix as substrate using Sporotrichum thermophile. [18]

IV. Figures

Figure 4.1 Screening for potential isolate

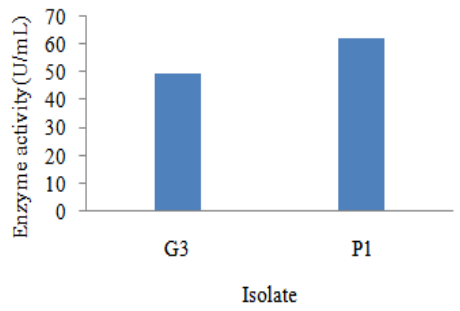


Figure 4.2 Effect of Incubation time

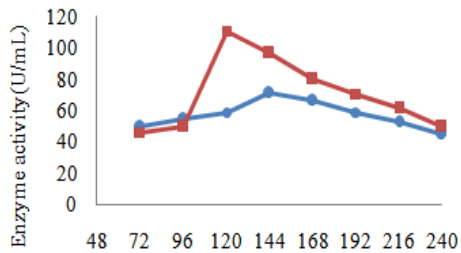


Figure 4.3 Effect of Temperature

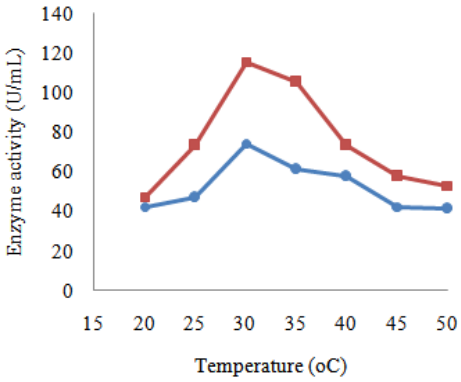
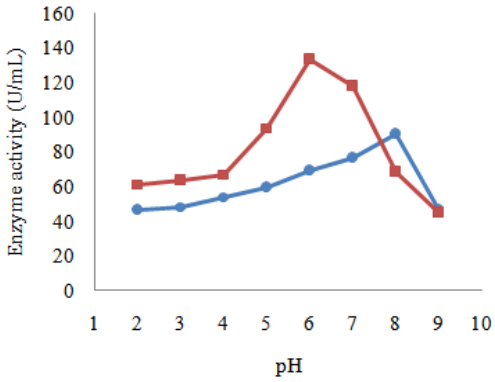
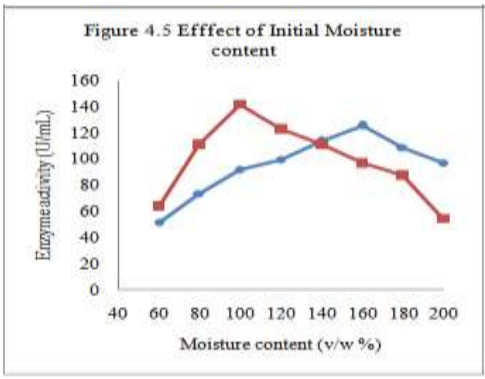
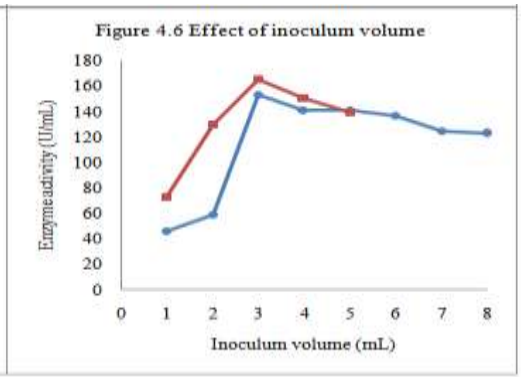
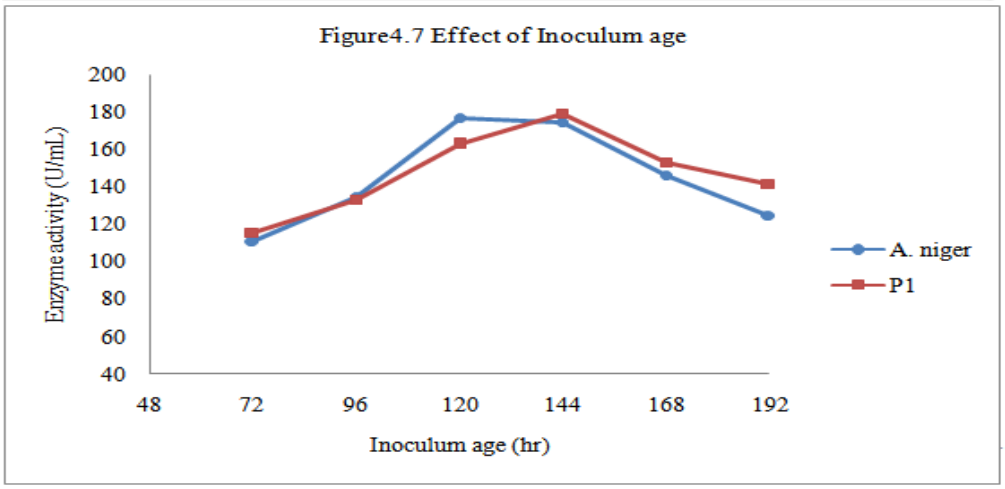


Figure 4.4 Effect of pH









V. Conclusion

The results obtained in the study showed that P1 isolate had more or less similar pectinolytic activity with respect to Aspergillus niger NCIM 616 by using guava waste as substrate in solid state fermentation. It is observed that the guava waste was found more effective substrate as it has high pectin content and supports growth of fungi which leads to high production of pectinase. It is commercially available at low cost and highly economical. P1 obtained from isolation is a promising source for the production of pectinase which used in many applications in food and textile industries. The optimization of process parameters were done for P1 and A. niger NCIM 616 are compared. The maximum pectinase activity of **176.8 U/ml** obtained after 144 hr of incubation, temperature of 30^{o}C, pH of 8, initial moisture content of 160 % and inoculum volume of 3 ml , inoculum age of 120 hr with Aspergillus niger NCIM. The maximum activity of **179.36 U/ml** obtained after 120 hr incubation time, temperature of 30^{o}C pH of 6, initial moisture content of 100 % and inoculum volume of 3 ml and Inoculum age 144 hr with P1 using guava waste powder as substrate

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