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Utilization of banana peel for pectinase enzyme production

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ABSTRACT

Banana peel, a fruit waste rich in pectin and other nutrients, was utilized in the present study to produce an industrially important pectinase enzyme. Banana peel powder as a sole source of carbon has been successfully exploited to grow *Aspergilus niger* (MTCC 281) under solid-state fermentation (SSF). The effect of quantity of nutrients, pH, temperature, and dilution level was studied on pectinase production. Optimizing the conditions for the production of strains and enzymes is necessary to use agricultural waste and properly attain sustainability of the bioprocess. Maximum pectinase activity of 8.12U/ml was observed at 1:3 dilution levels, pH 5, and 3% of 108 spores/ml inoculums size at 40°C with 120 hrs of incubation time. The current study addresses optimizing different cultural conditions for *A. niger* to maximize pectinase production with cheap and readily available banana peeling waste.

Keywords: *Musa* sp. Banana peel; pectinase production; optimization; *A. niger*

INTRODUCTION

In tropical nations, banana (*Musa* sp.) is one of the most widely available fruits. Global banana production is predicted to be 48.9 million tonnes, with India contributing 10.4 million tonnes. India is the world's biggest banana producer, followed by Brazil, Indonesia, the Philippines, China, and Australia. Except for the peel disposal, which generates more than 26 million tonnes of dry matter leftovers per year globally, all parts of the banana tree are beneficial to humans. Banana peel is a low-cost agricultural waste residue that is plentiful. It makes up around 40% of the weight of the raw fruit and is high in carbs, proteins, and a range of vitamins and minerals (Tibolla *et al.,* 13). Such by-products pose a complex question of waste management and further economic burdens on production. As banana peel is rich in pectin, therefore in order to reduce postharvest losses and increase the value of banana peel, it could be used as a substrate for pectinase production.

Pectinases are a family of enzymes that hydrolyze pectic compounds, primarily in plants. Cell wall expansion and softening of various plant tissues during maturity and storage are critical for plants. In the food industry, pectinases are considered one of the most effective hydrolytic enzymes and are used in foods of a pectinous nature to hydrolyze pectin and pectin-like colloids (Salim *et al.,* 10). Pectinases are also used in functional food production coffee and tea fermentation, oil extraction, juice clarification, pectic wastewater treatment etc. The enzyme can be sourced from microorganisms, plants and animals. Microbial pectinases account for 10% to 25% of global food and industrial enzyme sales and marketing. Because fungi are powerful producers of pectic enzymes, the majority of enzymes used in the food business come from them. Pectinases from the *Aspergillus* are most commonly employed in businesses because their metabolites are GRAS (Generally Recognized As Safe) (Reginatto *et al.,* 9). Solid-state fermentation (SSF) and submerged fermentation are important fermentation methods used for microbial enzyme production. Multiple environmental factors have an impact on pectinase production like nutrient concentration, pH, temperature, dilution level etc. The present study therefore, explores the optimization of different parameters under SSF for the production of pectinase by *A. niger* with banana peel waste which is a pectin-rich, low-cost, and readily available substrate.

MATERIALS AND METHODS

The fungal culture *Aspergillus niger* (MTCC 281) was obtained from Institute of Microbial Technology, Chandigarh, India. Culture was revived on potato dextrose agar (potatoes infusion from 200g; dextrose: 20g and agar: 20g per 1000ml) medium at 30°C for 7days. The culture was maintained and stored on PDA slants and sub-cultured after every 30 days. Banana (*Mussa spp.*) peel was collected from the local market of Hisar, India. Banana peel was dried at 45±2°C for 24 hours and grounded to form powder and passed through 100 micron mesh sieve. The yield of banana peel powder was 86%. The sieved banana peel powder (BPP) was stored at room temperature

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(25±2°C) and used as substrate by *A. niger* (MTCC 281) for pectinase production under solid state fermentation.

The fungal culture *A. niger* was inoculated on potato dextrose agar (PDA) medium supplemented with 2% pectin at pH 5 and incubated at 30°C for 5 days. To prepare homogenized spores suspension ten ml of sterilized distilled water was added to a 5 days old culture. An inoculum needle was used to dislodge the spore clusters under aseptic conditions and then it was shaken thoroughly. From the resulting suspension, 1ml of suspension (1×10^8 spores/ml) was used as inoculum for optimization of culture parameters (Dhillon *et al*., 3).

Experiments were carried out in 250 ml Erlenmeyer flasks containing 25g banana peel powder as sole carbon substrate supplemented with mineral solution containing KH_2PO_4 (0.02% w/v), $MgSO_4$ (0.01% w/v), $(NH4)_2SO_4$ (0.04% w/v), $FeSO_4$ (0.01% w/v), $MnSO_4$ (0.001% w/v)] in distilled water (Barman *et al*., 2) at pH 5 in triplicate. The contents were autoclaved at 121°C for 20 min and inoculated aseptically with 10^8 fungal spores after cooling. Inoculated flasks were incubated at 30°C for 5 days. Crude enzyme extract was harvested by centrifuging at 10,000 rpm for 15 minutes. The following cultural conditions were standardized for maximum pectinase production by *A. niger* using BPP as substrate.

The effect of dilution level on the pectinase production was studied by varying the substrate-to-dilution with sterilized distilled water (w/v). Erlenmeyer flasks (250 ml) with BPP as substrate moistened with 50 ml mineral medium in ratios (w/v) of 1:1, 1:2 and 1:3(banana peel powder : sterilized distilled water). The contents were autoclaved at 121°C for 20 min and inoculated aseptically with 10^8 fungal spores after cooling. Inoculated flasks were incubated at 30°C for 5 days. Crude enzyme was harvested by centrifuging at 10,000 rpm for 15 minutes and assayed for enzyme activity to determine the optimum dilution level for pectinase production.

To study the effect of pH, banana peel powder with (1:3) dilution level having inoculum of 10^8 spores/ml of *A. niger* was incubated at pH 4, 5 and 6 under shaking condition (200 rpm) at 40°C. Banana peel powder with inoculums of 10^8 spores/ml of culture @ 1, 2 and 3% incubated at 30°C with shaking at 200 rpm for 120 hours. Banana peel powder incubated at varied temperatures of 35°C, 40°C and 45°C and time ranging from 72, 96 and 120 hours. In each set of experiment the contents of the flasks were then harvested and assayed for enzyme activity. Enzyme activity was determined in crude enzyme extract for each treatment at specific stages of SSF fermentation. Twenty five ml of sterile distilled water was added into each flask containing fermented mash and placed on a shaker at 200 rpm for 60 min. Cheesecloth and Whatman No. 1 filter paper were used to filter the mixture, which was then centrifuged for 10 minutes at 10,000 rpm. The culture filtrate was collected and enzyme activity was measured (source of pectinase). Miller's approach was used to evaluate pectinase activity by monitoring the release of reducing sugars during the enzyme substrate reaction (Miller, 7). To analyze the effect of various factors the data were analyzed using ANOVA with factorial completely randomized design.

RESULTS AND DISCUSSION

Optimization of strains' growth and enzymes' production conditions is necessary for an appropriate use of agricultural waste and achieves sustainability of the bioprocess. In this study, *A. niger* was used to optimise pectinase synthesis using a solid state fermentation (SSF) process using BPP as the sole carbon substrate. Nine percent moisture, 8.6 percent ash, 4.8 percent protein, 7% fat, and 43.2 percent total dietary fibre were found in banana peel powder. Zaini *et al*. (14) also observed similar composition of banana peel powder. Dilution level, pH, inoculum size, temperature, and incubation time were all used to optimise fermentation conditions for optimum pectinase synthesis. With a 1:3 dilution level, pH 5, three percent inoculum of 10^8 spores/ml, maximum pectinase activity of 8.12 U/ml was recorded after 120 hours of incubation at 40°C.

Reginatto *et al.* (9) observed that production of pectinase by *A. niger* LB-02-SF is strongly affected by the medium composition as well as the delayed addition of pectin to the fermentation broth. The data presented in (Table 1) revealed that there were significant differences in pectinase activity at different dilution levels.

Maximum pectinase enzyme activity 3.43 (U/ml) in 1:1(w/v) dilution level, 3.79 (U/ml) in 1:2 (w/v),

Table 1. Effect of BPP: mineral solution dilution level on pectinase activity under SSF.

Treatment dilution level (w/v)	Enzyme activity (Unit/ml)			Mean
	Incubation time (h)			
	72	96	120	
1:1	2.62	3.18	3.43	3.078
1:2	3.41	3.52	3.79	3.57
1:3	3.42	4.17	4.37	3.99
Mean	3.15	3.63	3.86	
C.D at 5%	Treatment (T)=0.102, Incubation (I)=0.102, T×I= 0.176,			

dilution level and 4.37 (U/ml) in 1:3 (w/v) dilution level after 120 h incubation time was recorded. Significant increase in enzyme activity was observed with increase in dilution level up to 120 h incubation time, under SSF. The 1:3 dilution level was optimum with 120 hrs incubation time for pectinase production among the variables tested. During the secondary growth phase, higher dilution level had an inhibitory effect on microbial growth and enzyme secretion. This is consistent with that stated by Iqbal *et al.* (4) who said higher dilution results in decreased porosity of the substrate leading to inhibition of fungal growth. Additionally, low moisture content induces a sluggish fungal enzyme release due to reduced nutrient solubility and low growth substrate swelling. For pectinase synthesis, Koser *et al*. (5) conducted a study to optimise moisture (percent w/w) ranging from (50-75 percent) to the growth-supported substrate, i.e. lemon peel waste. After 4 days of inoculation with *A. oryzae*, lemon peel waste fermented at 70% (w/w) dilution produced the most pectin lyase activity (4.85 U/mL), but when the dilution level increased to 70%, pectin lyase output decreased. At various pH levels, significant variations in pectinase activity were observed. After 120 hours of incubation, the enzyme activity was found to be 2.16 U/ml in pH 4, 4.71 U/ml in pH 5, and 2.58 U/ml in pH 6. There was a significant increase in enzyme activity at pH 5. It was 4.36 U/ml at 72 h, 4.56 U/ml at 96 h and 4.71 U/ml at 120 h incubation time (Table 2).

Therefore, pH 5 was taken as optimum for pectinase production. Koser *et al.* (5) showed that *A. terreus* NCFT 4692.10 had improved pectinase production at pH 5.0. Patil and Dayanand (8) reported that, under both submerged fermentation (SmF) and solid state fermentation (SSF) conditions, pH 5.0 is optimal for maximum production of endo- and exo pectinases from de-seeded sunflower head. However, when contrasted with SmF and SSF conditions, the effect of pH on the production of pectinase from the sunflower head was not significant. The activity of the enzyme declined gradually after pH 5. The decline in enzyme activity at higher pH may be due to preference for growth and metabolism of the fungi to lower pH. These findings are consistent with those of Thangaratham and Manimegalai (12), who reported that pectinase activity increased with pH-value increase but further pH-increase beyond 5 reduced pectinase activities.

Inoculum is an important factor in the fermentation cycle because spore accumulation can impede the growth and development of the culture organism. Growth media with 1:3 dilution level was inoculated with varying inoculum size of 1%, 2% and 3% of 10^8 spores/ml at pH 5. It was observed that enzyme activity 3.44 U/ml in 1%, 4.31 U/ml in 2% and 5.35 U/ml in 3% inoculum level after 120 h incubation time (Table 3).

There was a significant increase in enzyme activity 5.30 U/ml at 3% inoculum size. Therefore, 3% of 10^8 spores/ml of *A. niger was* taken as optimum for pectinase production with 1:3 dilution level after 120 h incubation time. This result is marginally close to that of Sethi *et al.* (11) who noted that 2% inoculum size was capable of generating maximum pectinase activity but with a significant difference from that of Dhillon *et al.* (3) who observed maximum enzyme activity at 5% inoculum when the fungus was grown on a semi-solid substrate (15%) and incubated at 30°C for 120 h. An increase in inoculum size from 5% showed a gradual decrease in enzyme activity reaching the lowest at 20% inoculum, possibly due to nutrient depletion in the fermented medium. Ahmed *et al.* (1) reported that pectinase maximum activity was observed with an inoculum size of 8%, while the amount of reducing sugar produced and the pH of the medium was 3.2 and 4.3 respectively. The presence of large amounts of reduced sugars has been observed to have inhibitory effect on pectinase production. The generation of pectinase

Table 2. Effect of pH on pectinase activity under SSF.

Treatment pH level	Enzyme activity (Unit/ml) Incubation time (hrs)			Mean
	72	96	120	
4	2.87	2.35	2.16	2.46
5	4.36	4.56	4.71	4.54
6	2.22	2.57	2.58	2.46
Mean	3.15	3.16	3.15	
C.D. at 5%	Treatment (T)=0.075 Incubation (I)=N.S T×I= 0.130			

Table 3. Effect of inoculums size on pectinase activity under SSF.

Treatment Inoculums size (%)	Enzyme activity (Unit/ml) Incubation time (hrs)			Mean
	72	96	120	
1	3.36	3.40	3.44	3.40
2	4.17	4.24	4.31	4.24
3	5.22	5.27	5.30	5.27
Mean	4.25	4.31	4.35	
C.D. at 5%	Treatment (T)=0.066 Incubation (I)=0.066 T×I= N.S			

increased with the rise in the size of the inoculum, but reached a limit of 9% and decreased at 12%. Koser *et al.* (5) reported that a maximal pectin lyase activity in experimental flasks inoculated with 5 mL of fungal inoculum was observed in SSF media with 5 g of lemon peel waste and inoculum density levels ranging from 1 to 6 ml. The activity of pectin lyase has been observed to increase with an increase in inoculum density level from 1 to 5 ml, but the trend in activity profiles decreases with inoculum density higher than 5 ml. The fact that the lower level of inoculum density (1-4 ml) is insufficient for the culture organism to support fungal growth, resulting in a longer lag period, possibly explain these observations. The decrease in activity with higher inoculum density, on the other hand, could be related to a faster depletion of the nutritional supply (Iqbal *et al*., 4). It is known that temperature influences the metabolic rate of the organism involved in the process, which in turn determines the product's amount. The fermentation medium was incubated at various temperatures namely 35°C, 40°C and 45°C up to 120 hr. Enzyme activity was observed as 3.859 U/ml in 35°C, 8.120 U/ml in 40°C and 1.593 U/ml in 45°C after 120 h incubation time (Table 4).

There was a significant increase at 40°C as compared to other temperatures. The pattern in pectinase production observed to increase with temperature rise to 12.120 U / ml at 40°C, the activity of the enzyme decreased steadily under SSF to 1.593 U/ml at 45°C after 120 h of incubation time. The decrease in higher temperature enzyme activity could be due to denaturation of enzymes. The optimum temperature for purified polygalacturonase activity was found at 40°C. Patil and Dayanand (8) recorded optimum temperature of 34°C in both SmF and SSF for maximum pectinase output.

In the present study maximum pectinase activity observed at incubation of 120 hours as compared to 72h and 96h of incubation with different parameters of ph, dilution level,, inoculums size and temperature (Table 1,2,3,4). Maller *et al.* (6) reported that maximum pectinase production by *A. niveus* was recorded at 96 h of incubation that is consistent with current findings. The data analyzed showed that pectinase production during the fermentation process was slowly increased and reached its maximum value after 120 hours of initial incubation.

Cultural conditions for the production of pectinase under solid state fermentation were optimized by *A.niger* using banana peel powder sole carbon substrates. Among the factors evaluated in this work, the maximum pectinase activity was recorded at 8.12U/ml with a dilution level of 1:3, pH 5, 3 percent inoculum of 10^8 spores/ml and an incubation length of 120 hours at 40°C. Banana peel could therefore possibly be used as a substrate for pectinase production under SSF, and the produced pectinase can be used in food processing. It will help in banana waste management with industrial profitability.

Table 4. Effect of temperature on pectinase activity at various incubation times.

Treatment temperature (°C)	Enzyme activity (Unit/ml)			Mean
	Incubation time (hrs)			
	72	96	120	
35	1.57	1.65	3.86	2.36
40	3.92	6.48	8.12	6.17
45	1.39	1.57	1.59	1.52
Mean	2.29	3.23	4.52	
C.D at 5%	Treatment (T)=0.085 Incubation (I)=0.085 T×I= 0.147			

AUTHORS' CONTRIBUTION

Execution of field/lab experiments and data collection (Mohammad Sadiq); Conceptualization of research and contribution of experimental material (Dr. Anju Kumari); Preparation of the manuscript (Rehema Joshua); Designing of the experiments (Dr. Rakesh Kumar); Analysis of data and interpretation (Kritika Rawat).

DECLARATION

The authors do not have any conflict of interest.

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