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Pectinase Production in Solid State Fermentation by *Aspergillus niger* Orange Peel as a Substrate

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ABSTRACT

Pectinase are widely used in food industry especially in the processing of fruits & vegetables since they decrease the viscosity & facilitate clarification of fruit juices & wines. The potential synthesis of pectinase is wide spread among the microbial group including bacteria & fungi. The increasing energy demand has been focused worldwide attention on the utilization of renewable agricultural & industrial wastes. Present work was undertaken with the isolation of Aspergillus niger from soil samples & screening for fermentative production of pectinase by using orange peel as substrate.

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INTRODUCTION

Pectinase is a general term for enzymes, such as pectolyase, pectozyme and polygalacturonase, commonly referred to in brewing as pectic enzymes. This breaks down pectin, apolysaccharide substrate that is found in the cell walls of plants [1]. They can be extracted from fungus such as *Aspergillus niger*. The fungus produces these enzymes to break down the middle lamella in plants so that it can extract nutrients from the plant tissues and insert fungal hyphae [1]. Over the years pectinase have been used in several conventional industrial processes, such as textile, plant fiber processing, tea, coffee, oil extraction, treatment of industrial wastewater, containing pectinacious material, etc. They have also been reported to work on purification of viruses and in making of paper [2].

MATERIALS AND METHODS

Media and Cultural Conditions

For isolation of *Aspergillus niger*three soil samples were collected from KIAS, KIMSDU, Maharashtra, India and serial dilution were prepared upto10^{-6} and 0.1 mL of this dilution was spread inoculated on sterile Sabourauds agar plates in triplicates. These plates were incubated at 28^0 C for 5 days. Isolate were identified morphologically as *Aspergillus niger* by using lactophenol cotton blue staining and maintained on Sabourauds agar slant. The slant were kept at 4^0 C and was sub cultured in every 3 to 4 week.

Production of Pectinase [3]

a) Preparation of Substrate

Orange peels were collected from a local juice centre and dried by sun drying and grinded to make 350-g fine powder.

b) Preparation of Inoculum and Fermentation

Aspergillus niger subcultured on Sabourauds agar slants was used to prepare the spore suspension. The spore suspension was prepared in 15 mL of 0.01% Sodiul lauryl Sulphonate (SLS) detergent solution having 10^8spore/mL. The 100 g substrate of peels was soaked in 5 mL of spore suspension and distilled water in a three different pans for solid state fermentation at 28^0 C for 6 day,with daily sprinkling of 5mL distilled water and after getting moldy growth, they were harvested.

c) Extraction of Enzyme

After solid state fermentation, the enzyme was extracted from fermented substrate by homogenously mixing the entire substrate with 10 mM sodium acetate buffer- pH 5.0 (75 mL in each pan). The biomass was separated by filtration through a whatmann filter paper No.1 and extracts were centrifuged at 6000 rpm for 15 min and the clear supernatant was used as the source of extracellular crude pectinase enzyme.

e) Enzyme assay

Pectinase activity of the crude enzyme was assayed by measuring the reducing sugars releasing from the action of pectinase on citrus pectin using Dinitrosalicyclic acid (DNSA) reagent.(Solis *et al.*, 1996).

f) Purification of Pectinase

The crude extract was subjected for partial purification by 70% saturation of Ammonium Sulphate precipitation & subjected to dialysis to remove surface ions. The enzyme precipitate was dissolved in the 10 mM Na- acetate buffer (pH 5.0)and it was assayed again to study extent of purification and then enzyme solution was dried at 4^0 C.

RESULTS AND DISCUSSION

Results of Cultural & Morphological Characteristics

Isolates were studied for their identification, colony characterization, The *Aspergillus niger* isolate with black spores, profused growth, foot cell at the base of conidiophores of mycelium, was used for further study.

The colony characteristics of the isolate are as per Table-1.

Pectinase Production by *Aspergillus niger*

Pectinase assay was done to determine the yield of pectinase by DNSA method. The result of fermentative yield of pectinase before and after partial purification. by *Aspergillus niger* are shown in Tables 2, 3 and 4.

Pectinase production potential of the isolate was determined by growing isolate in pans containing dried orange peel powder & monitoring the enzyme production after 5 days.It is evident froms tables that the crude enzyme contained 1.29 U/ml while after partial purification it was increased(concentrated) to 6.14 U/mL.almost 4.75 times increase in the activity.

Effect of pH, Temperature & Substrate Concentration on Partially Purified Pectinase Enzyme (Plummer)

i) pH the enzyme preparation was subjected to buffers of p^H 2,3,4,5,6,7,8,9,10 & 11 using acetate, phosphate, carbonate and tris buffers in the assay procedure and residual enzyme activity was measured. The enzyme stability was studied on the basis of extent of residual activity. The p^H at which maximum residual enzyme activity was found was taken as optional p^H for enzyme activity. It is evident from Table-5 that the enzyme activity increased from acidic pH2(4.44 u/mL) to pH 5(6.48 u/mL) and from pH 6 onwards decreased gradually, indicating pH 5.0 as optimal pH.

ii) Temperature: The enzyme assay was performed at temperatures 0,4,8,25,37,42,55,70 & 100^0C, the temperature at which maximum residual enzyme activity was obtained was taken optimized temperature for enzyme activity. It is evident from Table-6 that the enzyme activity increased from temperature 0^0 C(1.85 U/mL) to 25^0 C ((7.83 u/mL) and from temperature 37^0C onwards decreased gradually, indicating temperature 25^0 C as optimal temperature.

iii) Substrate concentration: The enzyme assay was performed by using different concentrations of pure pectin (1, 2--- 10µg/mL). It is evident from Table-7 that enzyme activity was increased from substrate concentrations of 1, 2, 3, 4 µg/mL and at the substrate concentration 5 µg/mL and onwards it was maximum and almost constant activity of enzyme (1.30 to 1.31 U/mL), indicating at 5.0 µg/mL substrate concentration maximum enzyme activity was there.

Thus pH5.0, 25^0 C and 5.0 µg/mL substrate concentration were optimum for the pectinase enzyme obtained from *Aspergillus niger* isolate.

Table1: Morphological characteristics of the isolate colony on Sabourads agar at 28^0C for 6 days incubation

Isolate	Size	Shape	Colour	Margin	Elevation	Opacity	Consistency	Spore colour	Substrate mycelium
An1	5mm	Irregular	Black	Irregular	Convex	Opaque	Dry	Black	+

Table 2: Results of standard glucose curve

Concentration of Glucose in µg/mL	O.D at 540 nm
1	0.49
2	0.052
3	0.54
4	0.62
5	0.63
6	0.68
7	0.68
8	0.65
9	0.67
10	0.78

Table 3: Assay results of crude & partially purified enzyme

	Enzyme O.D at 540 nm	
Tube type	**Crude enzyme**	**Partially purified enzyme**
RC	0.00	0.00
SC	0.1	0.1
EC	0.06	0.06
TC	0.08	0.08
Test	0.16	0.43

Table 4: Pectinase production by *Aspergillus niger*

Substrate orange peel	Average (three sets) Pectinase activity U/mL x dilution factor
Crude enzyme (1:2 diluted)	1.29
Partially purified enzyme (1:10 diluted)	5.74

Table 5: Result of effect of p^H on pectinase activity

p^H	**Enzyme activity (U/mL)**
2	4.44
3	5.37
4	5.74
5	**6.48**
6	4.85
7	1.81
8	0.5
9	0.11
10	0.00
11	0.0

Table- 6: Result of effect of temperature on pectinase activity

Temp ^{0} C	**Enzyme activity (U/mL)**
0	1.85
4	2.22
8	3.40
25	**7.83**
37	7.03
42	6.17
55	0.07
70	0.0
100	0.0

Table-7: Result of effect of substrate concentration on pectinase activity

Concentration of Substrate µg/mL	**Enzyme activity (U/mL)**
1	0.74
2	0.55
3	1.20
4	1.24
5	1.30
6	1.30
7	1.30
8	1.30
9	1.31
10	1.31

CONCLUSION

Enzyme activity was found to be 1.29U/mL for crude enzyme and 5.74 U/mL for partially purified enzyme. Optimum p^H was found to be 5 and optimum temperature is25^0C with optimum substrate concentration of 5µg/mL.With optimization of production conditions the commercially viable quantity of pectinase can be produced from *Aspergillus niger*isolate.

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