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ISOLATION PRODUCTION AND PURIFICATION OF PECTINASE FROM SOIL ISOLATE

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ABSTRACT

In recent years the use of enzymes in biotechnological processes has grown considerably. In food and related industries, the use of enzymes in upgrading efficiency, increasing the yields of extractive processes, stabilizing the commodity, and enhancing the use of flavor and by-products was added great importance. Pectinases or pectinolytic enzymes today form one of the commercial sector's upcoming enzymes. Microbial pectinases have been reported to account for 25 per cent of global food enzyme sales. Bacteria were isolates from thirty coffee pulp samples were identified in the present investigation. The isolates were classified as actinomycete (21.06 percent), bacteria (65.26 percent), and fungi (13.68 percent), based on classification of the selective growth media. For these, 31.58 percent had colonies surrounded by clear zones that suggest pectinase development.

Key words: Pectinase, Polymeric, Pectin, Ingredient

INTRODUCTION

Pectin was first discovered in 1820 and has been found to be the primary ingredient in the manufacture of jam and jellies. Pectin is the central heteropolysaccharide portion of the primary cell wall of the whole plant on the substrate **[1,2]**. This contains a number of galacturonic acids. Pectin is a polymeric substrate with a carbohydrate component that is esterified with methanol. It is found in the middle lamella at a higher concentration **[3]**. Such enzymes are important for the degradation of dead plant material by pathogenic microorganisms and thus contribute to the recovery in the biosphere. Peptic product consists of protopectins, pectin acid, pectin's pectic acid **[4]**. The main chain of pectin is partly methyl esterified (1,4) d galacturonase **[5,6]**. De methylated pectin is known as pectin acid (pectate) or polygalacturonic acid. Pectinase (E.C. 3.2.1.15) is a family of hydrolases capable of hydrolysing pectin. The prevalling composition of pectin is homopolymeric, consisting of partly methylated– α (1, 4) galacturonic acid. The segment of – α (1, 2) –l –rhamnosyl – α (1, 4) – d galacturonsyl, comprising the branch points of l- arabinose and d galactose, can be integrated into the central polymeric strand. Pectin can contain residues of d – glucturnic acid, d – apiose, d – xylose and l – fucose attached to poly –α – (1, 4) – d galacturonic acid section. Pectic enzyme based on its attack on the galacturonase backbone of the pectin substance molecule **[7-9]**. A test is conducted for bacterial pectinase and its effectiveness in the bio-pulping of paper mulberry bark **[10]**. Twelve strains provide promising results for pectinolytic activity. Efficacy of bacillus sp. performed a screening of pectinase producing bacteria and their efficiency in bio pulping of paper mulberry bark. 12 strains give positive result for pectinolytic activity **[11]**. Efficiency of *bacillus sp*. Pectinase was analysed in the bio pulping of paper mulberry bark **[12].** Vibha *et al.,* 2010, have shown that pectinolytic microorganisms are widely distributed in soil, spoiled fruits, plants decayed leaves and wood as well as in water samples taken from rotting coconut, husk particularly in coastal areas.168 bacterial strains capable of developing citrus pectin-containing medium as the only carbon source were isolated [**13-15]**.

These strains were graded as very good pectinase producers when viewed as a strong zone around the colonies of at least 1.5cm, a strong producer when the zone was at least 1 cm, a bad producer when the zone was at least 0.5 cm and a marginal producer when there was no pectin-containing production and no clear region.

METHODOLOGY

Collection of sample:

Three separate soil samples were obtained from various places in Raibareilly UP, the source chosen for the sample collection is dependent on the presence of the pectin there **[16]**.

Isolation of bacteria from soil sample:

Bacterial cultures were isolated from the sample by serial dilution and spread plate method. Such cultures were first transformed to pure cultures by streaking and then screened for pectinase production using minimal agar salt media supplemented with 1% pectin as a substrate **[17,18]**.

Biochemical screening for culture 5:

Biochemical analysis was carried out to classify the strain **[19]**.

Selection of production media:

The selection of production media was carried out by observing the bacterial growth in minimum three different media. It implies that the overall growth is equal because the average OD is present in the presence of medium **[20]**.

Fermentation and purification of pectinase:

The selective medium was prepared and the culture was inoculated and held for fermentation at 37°C for 1 week at 100 revolutions per minute. The purification of the pectinase was carried out by implementation of salt precipitation using 40% ammonium sulphate and dialysis **[21]**.

Estimation of pectinase:

The measurement of pectinase was conducted by DNS assay **[22]** using pectin as a substrate and Lowry's method for the calculation proteins concentration **[23]**.

RESULTS AND DISCUSSIONS

Sample collection:

Three different samples were collected from different location mentioned in table

Table 1: list of the soil samples we collected from different places.

S no.	Colour	Sample
1	Brown	Coconut waste 
2	Brown	Public Park 
3	Blackish	Fruit waste 

Isolation of bacteria from soil samples

Pectinase producing bacterial strain was isolated through serial dilution and spread plate method. Mixed culture plates were differentiated on the basis of morphological characteristics and named as akpv2017 01 to akpv2017 11.

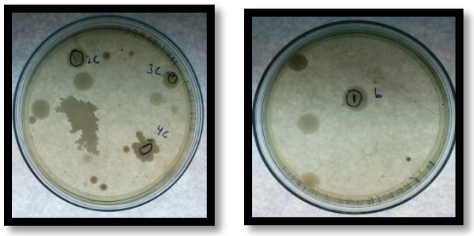

Figure 1: above figure represent the spreading of diluted soil sample

Figure 2: above figure represent the spreading of diluted soil sample 2

Figure 3: above figure represent the spreading of diluted soil sample 3.

Colony morphology

The colonies were differentiated on the basis of their morphology. The physical characterstics of the colonies such as colony, shape, margins, elevation, surface, pigmentation, texture and opacity were studied.

Table 2: colony morphology of different bacterial colonie

Culture	Shape	Margin	Texture	Surface
01	Filamentous	Discrete	Soft	Smooth
02	Circular	Entire	Soft	Smooth
03	Circular	Entire	Soft	Smooth
04	Circular	Entire	Soft	Smooth
05	Circular	Entire	Soft	Smooth
06	Irregular	Discrete	Soft	Smooth
07	Rhizoidal	Discrete	Soft	Smooth
08	Rhizoidal	Discrete	Soft	Smooth
09	Circular	Lobate	Soft	Smooth
10	Rhizoidal	Discrete	Soft	Smooth
11	Rhizoidal	Discrete	Soft	Smooth

Culture	Elevation	Pigmentation	Opacity
01	Raised	Cream	Opaque
02	Raised	Cream	Opaque
03	Raised	Cream	Opaque
04	Raised	Cream	Opaque
05	Raised	Cream	Opaque
06	Umbonate	Cream	Opaque
07	Raised	Off white	Opaque
08	Flat	Off white	Opaque
09	Raised	Cream	Opaque
10	Raised	Cream	Opaque
11	Raised	Cream	Opaque

Purification of mixed culture

Pure was obtained after streaking the selected colonies from the mother plate.

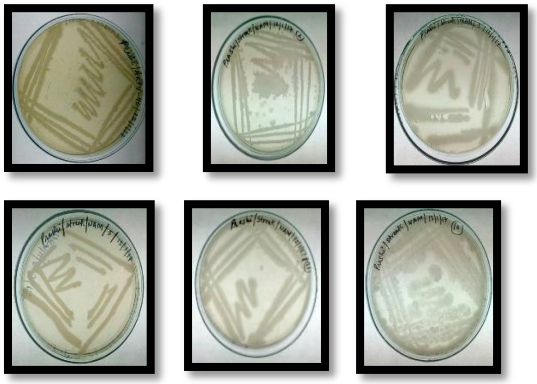

Figure 4: represent the purification of culture from mixed culture.

Primar and secondary screening for pectinase producing bacteria

Screening of pectinase producing bacteria was done by addition of iodine solution to the plates. Zone of hydrolysis was visualized on the plates. The maximium zone of hydrolysis was shown in akpv2017 05.

Table 3: results of primary and secondary screening of purified culture

Culture number	Primary screening	Secondary screening
1	+	_
2	_	_
3	+++	_
4	+++	_
5	+++	++
6	++	+
7	+++	_
8	+++	+
9	_	_
10	+	_
11	+++	+

Identification and biochemical test for the culture

The culture akpv2017 05 showing maximum zone of hydrolysis was selected for further studies and was identified by performing and comparing various staining and biochemical tests.

Table 4: staining and biochemical tests of **akpv2017 05.**

s.no.	test	result
1	gram staining	+ *Bacillus*
2	endospore staining	+
3	catalase	+
4	mannitol test	+
5	vp test	+

Selection of production media

Production media was selected for the best growth of our isolate so it produce maximum amount of enzyme. Hence, for this six production media was formed . To check the best media for the growth of isolate dns was performed for successive 24 hours.

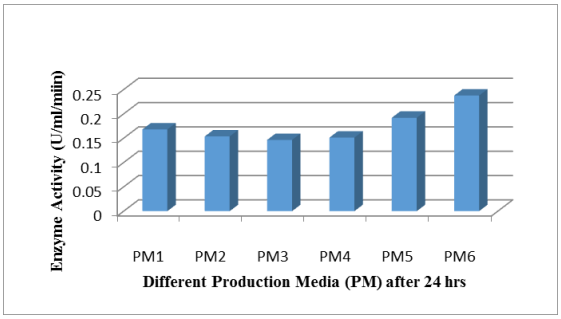

Figure 5: above graph represent different production media after 24 hrs vs enzyme activity, that shows in different production media pectinase produce in different quantity and best production occurred in pm6.

Fermentation and purification of pectinase produced by akpv2017 05

Fermentation was done by using shake flask in which they were grown in best production media under optimized condition for 7 days and then the crude enzyme was extracted by centrifugation. The crude enzyme was obtained from submerged fermentation was precipitated through ammonium sulphate and isoelectric point where as the enzyme ibtained from solid state fermentation was precipitated through isoelectric point. The precipitated enzyme was centrifused and the pellet was dissolved in the tris buffer solution. Dialysis is done to remove impurities and the salt from the enzyme.

Enzyme activity of pectinase enzyme

The dns assay was done in order to find out the activity of the different forms of such as crude salt pericipitated and pure enzyme obtained through submerged and solid state fermentation.

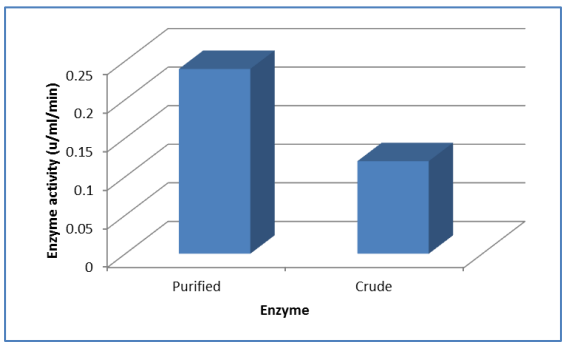

Figure 6: enzyme activity of pectinase after salt precipitation and dialysis by dns method.

Concentration of protein:

The Lowry's assay was done in order to find out the concentration of protein.

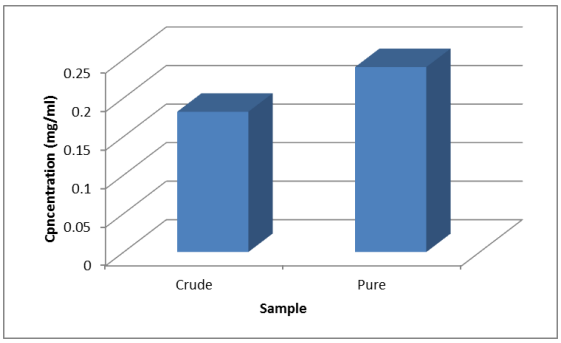

Figure 7: Graphical representation of concentration of pure and crude product.

CONCLUSION:

In this analysis, pectinase activity is calculated using the 5-dinitrosalicylate reagent method specifically to quantify the amount of reduced sugar by colorimetric methods. Based on pectinase assay procedures and properties, the pectinase of the isolates screened and classified in this study resembles polygalacturonase.

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