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ISOLATION, SCREENING AND IDENTIFICATION OF A POTENT PECTINOLYTIC BACTERIA FROM FRUIT WASTE

Short title: Pectinases from bacterial isolates

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Abstract: Pectinases constitute a unique group of enzymes which catalyze the degradation of pectic polymers present in plant cell wall. It digests a galacturonic acid polymer by breaking the α- 1,4 glycosidic linkages between the giant molecules. The majority of studies on pectinases have focused on fungi, with fewer studies on bacterial pectinases than on fungal pectinases. Pectinases from bacteria exhibit several advantages over fungal pectinases. As a result, the goal of this study was to isolate and screen pectinase-producing bacterial strains from fruit waste. In the end, 14 bacterial strains (SV-01 to SV-14) were identified, with SV-09 demonstrating the most pronounced hydrolysis zones around the bacteria colony in substrate-containing agar plates. The morphological tests on the chosen isolate (SV-09) revealed gram-positive, aerobic, motile rods with a whitish appearance.

Keywords **– Fruit waste, Bacteria, Screening, Food processing enzymes, Pectinase, Industrial applications**

I. INTRODUCTION

In the juice industry, the use of food processing enzymes like pectinase has increased dramatically [1,2]. Pectinase is an enzyme that has been used since ancient times and was first used commercially in 1930 for the manufacture of wines and fruit juices [3]. In general, enzymes are often used in food and fruit processing sectors to improve product quality while minimizing overall production costs [4]. Enzymes are biocatalysts that speed up and catalyze a variety of biological reactions [5]. Depectinization enzymes, such as pectinase enzyme, are used in the fruit juice industry to clarify the juice [6]. Pectin is an acidic heteropolysaccharide found in the plant cell wall's middle lamella and is a negatively charged high molecular weight heteropolysaccharide made up of a main chain of D-galacturonic acid monomers linked by (1->4) linkages. pectic substances are got from middle lamella of cell walls of plants in conjunction with cellulose [7]. They are group designated for colloidal carbohydrate derivatives and they function to move H_2O and cement materials for the cellulose network [8]. Pectin gives structural integrity to the cells and make them cohesive [9]. There are three major groups of pectic substances, all of these contains D-galacturonic acid as the common component which may be in lesser or greater extent depending upon the group [10] . The three major groups of pectic substrates include: homogalacturonan (HG), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II) (Figure 1).

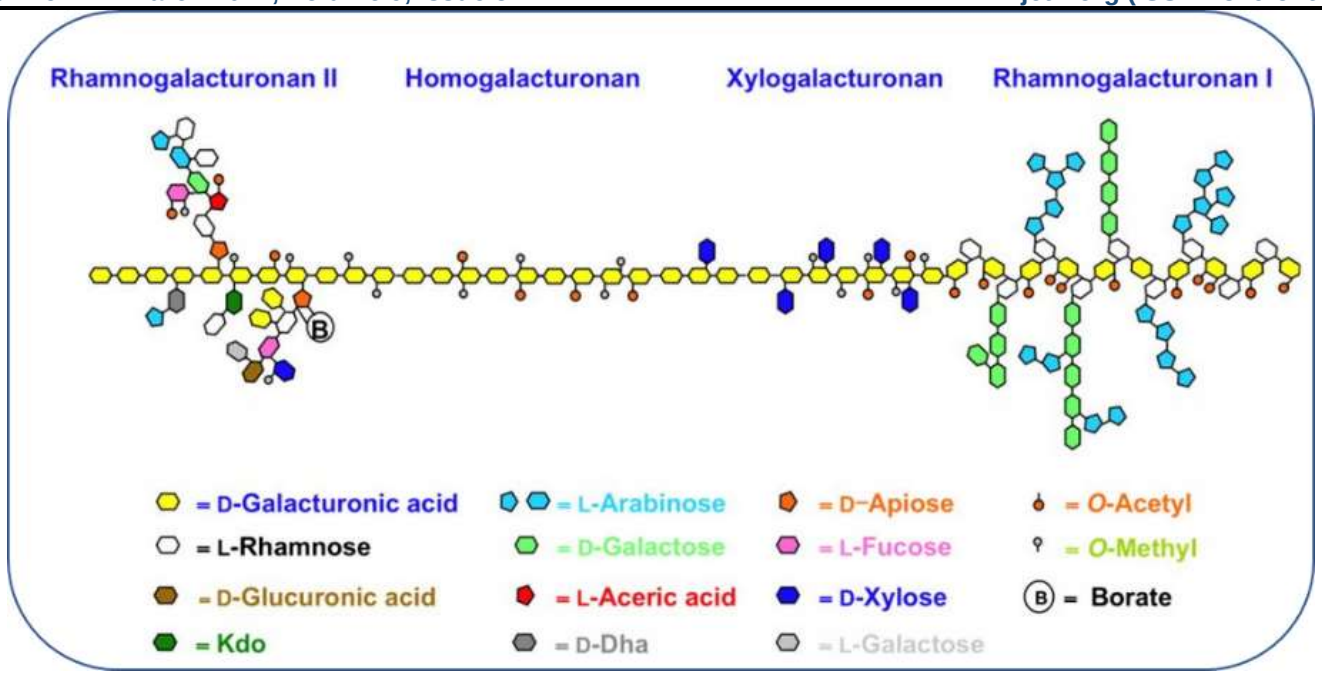

Figure 1. The three major groups of pectic substrates

Homogalacturonans are the linear polymers of pectins, which are formed by D-galacturonic acid monomers, which may be methyl esterified and/or acylated [11]. This region of pectin is called smooth region. These molecules are further classified on the basis of esterification level or carboxylic groups. If 75% of carboxylic acids are methylated then it is called pectin, if less than 75% of carboxylic acids are methylated the substances are called pectic acids and if there is no methyl esterification of carboxylic acid it is called polyglacturonic acid [12]. This pectin polymer is broken down into monomer sugars, such as galacturonic acid, using pectinases [13]. Pectinase enzyme has a wide range of applications in diverse of industries. In the fruit juice industry, pectinases are used to increase the quality of juice by digesting fruits and vegetables [14]. For maceration of fruit tissue in the wine business, as a macerating enzyme on vegetables to extract oil that is used for a variety of uses ranging from aromatic items to cosmetics and detergents [15]. Pectinases are enzymes that are utilized to produce flavour in a variety of foods [16]. To minimize viscosity and mucilage, they are used in coffee and instant tea processing [17]. Pectic enzymes are divided into two types based on the optimum pH for enzyme activity: acidic and alkaline pectinase. Acidic pectinase has a longer history of research and application than alkaline pectinase [18]. Fungi are the primary source of acidic pectinases, while alkalophilic bacteria, produce alkaline pectinases. Furthermore, bacterial pectinases are frequently stable over a wide temperature and pH range, but fungal pectinases are less stable at high temperatures and pH levels [18]. As a result, bacterial pectinases can be used in a variety of industrial applications that need a high temperature and/or pH. The alkaline pectinase has developed as important commercial enzymes with far-flung applications [17]. Many researchers are highly focused on the possible applications and uses of microbial alkaline pectinases, the nature of pectin, and the wide range of pectinolytic enzymes that work to mineralize pectic compounds found in the environment. It also underlines the environmentally friendly applications of microbial alkaline pectinases, revealing their unappreciated potential. As a result, the purpose of this study was to find an efficient pectinolytic bacterium and use it to produce increased pectinase.

II. Materials and Methods

Sample collection and processing:

For the isolation of pectinase-producing bacterial strains, a total of nine samples were collected from six distinct locations, including fruit and vegetable wastes, decaying coconut husks, and agricultural waste. Nutrient pectin agar was used for screening of pectinolytic bacteria.

Qualitative screening for pectinase producing bacteria:

For qualitative screening of pectinase producing bacteria, the isolated bacterial colonies were streaked onto nutrient pectin agar media plates. The plates were incubated at 37°C for 24 h and stained with I2/KI solution (0.5% I2 dissolved in 1% KI solution) for 30 min followed by destaining with distilled water. The pectinase producing bacteria were identified by the formation of yellow zone of hydrolysis against dark brown background.

Quantitative screening for pectinase-producing bacteria:

The bacterial isolates that produced pectinase under submerged fermentation in 250 mL Erlenmeyer flasks containing 50 mL Horikoshi media were subjected to quantitative screening for pectinase quantification.

The flasks were inoculated with overnight grown cultures at 1% level and incubated at 37°C for 48 h in an orbital shaker at 200 rpm. After incubation, the flasks were taken out and the culture filtrates were centrifuged at 10,000 x g for 15 min at 4°C. The resulting clear supernatants were collected and assayed for pectinase activity.

Assay of pectinase activity:

Pectinase activity was assayed by measuring the amount of D-galacturonic acid liberated from pectin by the enzyme. One unit of pectinase activity was defined as the amount of enzyme required to liberate 1 µmol of D-galacturonic acid/min under the assay conditions

Protein estimation:

Protein concentration was estimated according to Bradford's method [19]

Identification of the selected pectinase-producing bacterial isolate

On the basis of qualitative and quantitative analysis of the isolates, a potent pectinase producing bacterial isolate (SV-09) was selected for further studies. Morphological and biochemical tests were performed on the selected bacterial isolate.

III. RESULTS AND DISCUSSION.

Isolation and screening of bacterial isolates for pectinolytic activity

From the collected samples, 14 bacteria (SV-01 to SV-14) were successfully isolated. The isolate was purified using the streak plate method and quantitatively screened using the plate assay method. The preliminary screening for pectinase was performed using an iodine test and verified using Cetyl trimethyl-ammonium bromide (CTAB), providing 9 positive results (Table 1). Different substrates have been utilised in the media used to isolate pectin-degrading bacteria by researchers. The most common substrate for the isolation of pectinolytic bacteria is pectin. Commercial pectin, on the other hand, is prohibitively expensive for isolating pectinolytic bacteria. Fruit peel wastes, such as mango peel, mosambi peel and pomegranate peel, are high in pectin and can thus be used to screen bacteria that produce pectinase. Mango peel has been employed for the first time to boost pectinase synthesis by the potent pectinolytic bacteria SV-09.

Quantitative analysis of pectinolytic bacterial isolates

All the 9 bacterial isolates showing a zone of hydrolysis on agar plates were quantitatively screened for the production of pectinase in SmF by using mango peel as a substrate. The isolates were grown in Horikoshi medium under unoptimized conditions. The production medium was inoculated with 2% inoculum of overnight grown bacterial culture and incubated at 37°C for 48 h in an orbital shaker at 200 rpm. The bacterial isolate SV-09 showed the highest pectinase activity (313.90 IU/GDS) followed by SV-04 (218.63 IU/GDS) and SV-05 (215.27 IU/GDS). The lowest pectinase activity of 106.32 IU/GDS was recorded for the isolate SV-03. With a few exceptions, the pectinase activity generally corresponded to the size of zone of hydrolysis (Figure 2).

Table 1. Screening of pectinase producing bacterial isolates

S. No	Isolate	Zone size (CM)	S. No.	Isolate	Zone size (CM)
1.	SV – 01	1.0	8.	SV – 08	--
2.	SV – 02	--	9.	SV – 09	2.3
3.	SV – 03	1.8	10.	SV – 10	1.8
4.	SV – 04	2.1	11.	SV – 11	--
5.	SV – 05	1.2	12.	SV – 12	1.4
6	SV – 06	--	13.	SV – 13	1.2
7.	SV – 07	1.7	14.	SV – 14	--

Figure 2. Zone of substrate hydrolysis shown by the potent pectinolytic isolate, SV-09.

Table 2. Quantitative analysis of bacterial isolates for pectinase production

Isolate	Pectinase Activity (IU/GDS)	Isolate	Pectinase Activity (IU/GDS)	Isolate	Pectinase Activity (IU/GDS)
SV-1	112.45	**SV-5**	215.27	**SV-10**	114.52
SV-3	106.32	**SV-7**	209.45	**SV-12**	112.23
SV-4	218.63	**SV-9**	313.90	**SV-13**	207.54

Identification on the basis of morphological, physiological and biochemical characteristics

Morphological, physiological and biochemical characteristics of SV-09 isolate have been presented in Figure 3 and Table 3. The morphological tests of the selected isolate revealed that the bacterial cells were whitish in colour, gram-positive, aerobic, moderately sized and motile rods. The biochemical testing of the selected isolate revealed that the bacterial strain was positive for catalase activity and hydrolysis of pectin but negative for starch hydrolysis (Table 3). On the basis of morphological, physiological and biochemical tests, the selected strain was identified as *Bacillus* sp. and designated as *Bacillus* sp. SV-09.

Figure 3. Morphological, physiological and biochemical characteristics of SV-09

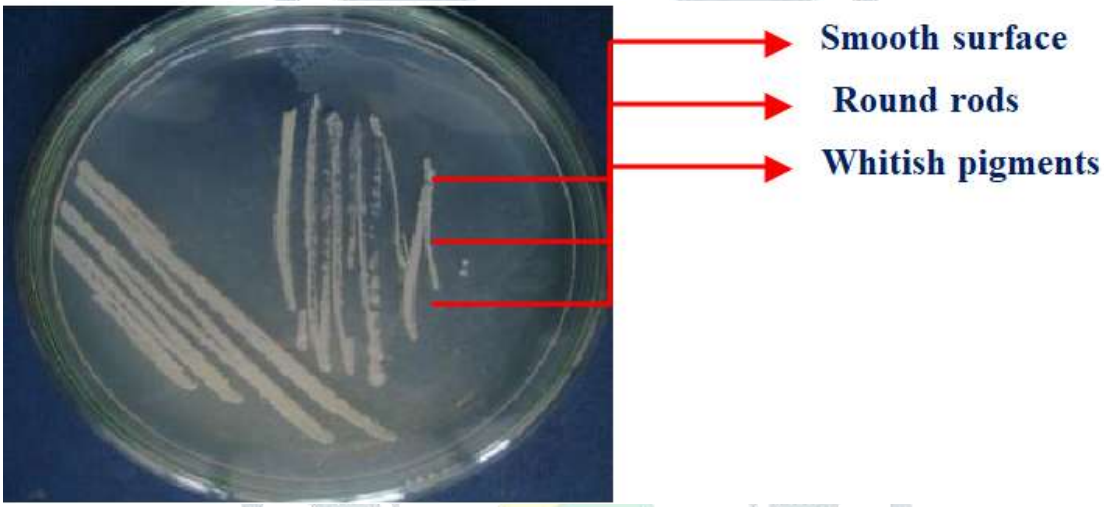

Table 3. Biochemical characteristics of the selected bacterial isolate SV-09.

TEST	RESULT	TEST	RESULT	TEST	RESULT
Citrate utilization	Positive	**Catalase test**	**Positive**	**H_2S production**	Negative
Gelatin hydrolysis	Positive	**Pectin hydrolysis Test**	**Positive**	**Gas production from glucose**	Negative
Nitrate reduction	Positive	**Starch hydrolysis**	Negative	**Gram's stain**	Positive

IV. SUMMARY AND CONCLUSION

Pectinases are a special class of enzymes that catalyse the breakdown of pectic polymers found in plant cell walls. It breaks the -1,4 glycosidic connections between the large molecules to digest a galacturonic acid polymer. The majority of pectinase research has concentrated on fungi, with bacterial pectinases receiving far fewer studies than fungal pectinases. Bacterial pectinases have various benefits over fungal pectinases. As a result, we intended to collect pectinase-producing bacterial strains from fruit waste and other biowaste and screen them. In the end, 14 bacterial strains (SV-01 to SV-14) were identified, with SV-09 exhibiting the most prominent hydrolysis zones around the bacteria colony in substrate-containing agar plates. In morphological testing, the chosen isolate (SV-09) showed gram-positive, aerobic, motile rods with a whitish appearance. Molecular techniques are being used to identify the potent bacteria, as well as to optimise the cultural and nutritional parameters for increased pectinase production by the SV-09 strain.

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REFERENCES

[1] Youn KS, Hong JH, Bae DH, Kim SJ, Kim SD (2004). Effective clarifying process of reconstituted apple juice using membrane filtration with filter-aid pretreatment. Journal of Membrane Science. 228(2):179-86.

[2] Gengatharan A, Dykes G, Choo WS (2021). Betacyanins from Hylocereus polyrhizus: pectinase-assisted extraction and application as a natural food colourant in ice cream. Journal of Food Science and Technology. 58(4):1401-10.

[3] Amin F, Bhatti HN, Bilal M (2019). Recent advances in the production strategies of microbial pectinases—A review. International journal of biological macromolecules. 122:1017-26.

[4] Bilal M, Iqbal HM (2020). State-of-the-art strategies and applied perspectives of enzyme biocatalysis in food sector—current status and future trends. Critical reviews in food science and nutrition. 60(12):2052-66.

[5] Robinson PK (2015). Enzymes: principles and biotechnological applications. Essays in biochemistry. 59:1.

[6] Alvarez S, Alvarez R, Riera FA, Coca J (1998). Influence of depectinization on apple juice ultrafiltration. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 138(2-3): 377-82.

[7] Parker CC, Parker ML, Smith AC, Waldron KW (2001). Pectin distribution at the surface of potato parenchyma cells in relation to cell− cell adhesion. Journal of agricultural and food chemistry. 49(9): 4364-71.

[8] Zuber M, Zia KM, Barikani M (2013). Chitin and chitosan based blends, composites and nanocomposites. InAdvances in natural polymers (pp. 55-119). Springer, Berlin, Heidelberg..

[9] Ochoa-Villarreal M, Aispuro-Hernández E, Vargas-Arispuro I, Martínez-Téllez MÁ (2012). Plant cell wall polymers: function, structure and biological activity of their derivatives. Polymerization. 4:63-86.

[10] Yapo BM (2011). Pectic substances: From simple pectic polysaccharides to complex pectins—A new hypothetical model. Carbohydrate Polymers. 86(2): 373-85.

[11] Nunes C, Silva L, Fernandes AP, Guiné RP, Domingues MR, Coimbra MA (2012). Occurrence of cellobiose residues directly linked to galacturonic acid in pectic polysaccharides. Carbohydrate polymers. 87(1): 620-6.

[12] Tabssum F, Ali SS (2018). Screening of Pectinase Producing Gram Positive Bacteria: Isolation and Characterization. Punjab University Journal of Zoology. 3(1):11-5.

[13] Edwards MC, Henriksen ED, Yomano LP, Gardner BC, Sharma LN, Ingram LO, Doran Peterson J (2011). Addition of genes for cellobiase and pectinolytic activity in Escherichia coli for fuel ethanol production from pectin-rich lignocellulosic biomass. Applied and Environmental Microbiology. 77(15): 5184-91.

[14] Garg G, Singh A, Kaur A, Singh R, Kaur J, Mahajan R (2015). Microbial pectinases: an ecofriendly tool of nature for industries. 3 Biotech. 6(1):1-3.

[15] Verma H, Narnoliya LK, Jadaun JS (2018) . Pectinase: a useful tool in fruit processing industries. Nutr Food Sci Int J. 5(555673):10-9080.

[16] Couto SR, Sanromán MA (2006). Application of solid-state fermentation to food industry—a review. Journal of Food Engineering. 2006 Oct 1;76(3):291-302.

[17] Mojsov KD. Aspergillus enzymes for food industries. Innew and future developments in microbial biotechnology and bioengineering 2016 Jan 1 (pp. 215-222). Elsevier.

[18] Nighojkar A, Patidar MK, Nighojkar S (2019). Pectinases: production and applications for fruit juice beverages. InProcessing and Sustainability of Beverages (pp. 235-273). Woodhead Publishing.

[19] Ninfa AJ, Ballou DP, Benore M. Fundamental laboratory approaches for biochemistry and biotechnology. John Wiley & Sons; 2009 May 26.