The Academic
International Journal of Multidisciplinary Research

An Online Peer Reviewed / Refereed Journal
Volume 2 | Issue 8 | August 2024
ISSN: 2583-973X (Online)
Website: www.theacademic.in

Screening and Production of Pectinase from Microbial Sources and Determination of its Enzyme Activity

Toshavi Kolhe[1], Sukrut Vyawahare[2] and Aman Tiple[3*]
[1]Institute of Biosciences and Technology, Chh. Sambhajinagar, Maharashtra, India
[2]Amrita School of Biotechnology, Amritapuri, Kollam, Kerala, India
[3]Vasishth Genomics Research Lab, Bengaluru, Karnataka, India
[*]Email: amantiple97@gmail.com

ARTICLE DETAILS

Research Paper

Keywords:

Pectin, Pectinase, Aspergillus niger, Enzyme activity, Banana

ABSTRACT

Pectinases are group of enzymes is responsible for catalysing the breakdown of pectic materials. It can be used extensively in the food industry to produce and clarify juices and wines. Higher plants' primary cell wall and middle lamella both contain significant amounts of pectin. Acidic heteropolysaccharide with a high molecular weight, pectin is mostly composed of α (1−4) linked d-galacturonic acid residues. There are three main kinds of pectic polysaccharides that have been identified, and they are all partially or mostly composed of d-galacturonic acid. Rhamnogalacturonan I (RGI), rhamnogalacturonan II (RGII), and homogalacturonan (HG) are their names. A class of enzymes known as pectinases is categorised based on how they break down pectic substances. These enzymes are helpful for extracting fruit juice, clarifying wine, concentration and fermentation of tea, cocoa, and coffee, extraction of vegetable oil, making jam and jellies, and pickling. Moreover, the paper and pulp industries, bleaching paper, bio-scouring cotton, retting and degumming plant fibres, oil extraction, wastewater treatment, additives for poultry feed, protoplast fusion technology, and the generation of bioenergy all make use of these enzymes. Pectinase is produced and secreted by yeast, fungus, and

bacteria. Extracellular enzymes that are readily retrieved are produced by the fungus *Aspergillus*, *Rhizopus*, and *Penicillium*. These enzymes are usually considered safe (GRAS). In this work, yeast and *Aspergillus Niger* digest bananas to create the pectinase enzyme. The enzyme that is generated is utilised to clarify juice.

1. Introduction:

Enzymes are substances present in the cells of living organisms in small quantities which are able of speeding up chemical responses without themselves being altered after the response. As compared to chemical catalysts, enzymes have numerous advantages similar as high particularity, a high catalytic effectiveness, and a malleable exertion, which greatly promote the use of enzymes in medicinal, chemical, and food diligence (Pires- Cabral et al, 2010). Due to these desirable features, the demand for artificial enzymes has pelted to new heights which call for constant exploration and development, to optimize their product and minimize resource costs (Arnav J et al, 2020). The discovery of enzymes was started in the middle of the nineteenth century and they were first introduced in the artificial operation using fungal enzymes. Still, after twenty years Boidin and Effront introduced the bacterial enzymes in the industry (Tabssum F & Ali S.2018). Utmost of the artificial demand for enzymes has begun from microorganisms. Due to their high growing capability, short life span, and easiness of inheritable manipulation, microorganisms are preferred in industry for enzyme product. Microbial enzymes are supplied, well- formalized, and retailed by a many contending companies. Among these industrially important enzymes, pectinase have a special significance due to their multiple uses in important sectors similar as food, cloth, potables, pulp and paper, and biofuel diligence (Rombouts F & Pilnik W. 1980).

Pectin is an important element of the middle lamina and primary cell wall of advanced shops. Pectins are high molecular weight acidic heteropolysaccharide primarily made up of α (1 -4) linked d- galacturonic acid remainders. Three major pectic polysaccharides groups are honored, all containing d- galacturonic acid to a lesser or a lower extent. They're homogalacturonan (HG), rhamnogalacturonan I (RGI), and rhamnogalacturonan II (RGII). Pectinase are a group of enzymes that act upon pectic substance and are characterized according to their medium of action. For illustration, methylesterases remove methoxy groups from largely or incompletely esterified galacturonan. Polygalacturonases beget the hydrolysis of the glycosidic bonds in a arbitrary fashion (endo- polygalacturonases) or from the non-reducing end of

homogalacturonan releasing galacturonic or digalacturunic acid remainders (exopolygalacturonases). Pectinolytic enzymes, or pectinase, are also classified according to their mode of action and their substrate polygalacturonases, which are sub classified as endopolygalacturonases(E.C.3.2.1.15) and exo-polygalacturonases(E.C.3.2.1.67); lyases, which are sub classified into pectatelyases(E.C.4.2.2.9 and EC.4.2.2.2) or pectin lyases(E.C.4.2.2.10); and pectin methylesterases(E.C.3.1.1.11). It's recommended to use a combination of different kinds of pectinase, along with other enzymes similar as cellulases and hemicelullases, as multiple enzymes can degrade different corridor of the polymer, performing in the minimal declination of the pectin in colorful raw accoutrements similar as in citrus juice processing (Oumer O et al, 2018). Pectinase have pivotal places in food diligence. These enzymes are useful for fruit juice birth and wine explanation; tea, cocoa, and coffee attention and fermentation; vegetable oil painting birth; medication of jam and jellies; and pickling (Barman S et al, 2015). Likewise, these enzymes are used in paper and pulp diligence, bleaching of paper, bio-scouring of cotton, retting and degumming of factory filaments, oil painting birth, wastewater treatment, and flesh feed complements, protoplast emulsion technology, and bioenergy product.

Utmost pectic enzyme medications are used in the fruit processing industry and pectic enzymes alone account for about one quarter of the world's food enzyme product (Patil S et al, 2006). Bacteria, fungi and yeast produce and secrete pectinase (Rombouts F et al, 1980). Enzymes produced from the fungi *Aspergillus*, *Rhizopus* and *Penicillium* are generally regarded as safe (GRAS) and produces extracellular enzymes which can be fluently recovered. Various fruit and vegetable processing waste have been used for the product of pectinase (Bari M et al, 2010; Kumar Y et al, 2012). Bananas are the second most produced fruit globally, after citrus. Banana is substantially eaten fresh but it's also suitable for recycling into products (Lee W etal. 2006). They're substantially grown by small- scale growers and play an important socioeconomic part in numerous developing countries of the tropics and subtropics. Banana peel is an cheap and plentiful agricultural waste byproduct. It's fluently available in large amounts. It accounts for about 40 of the weight of the raw fruit and is rich in carbohydrates, protein and colorful vitamins and mineral rudiments (Gadge P & Dandu M, 2022). Bananas are generally too pulpy and tenacious to yield juice by normal hydraulic pressing or centrifugation (Lee W et al, 2006). Traditional mechanical system of pressing, folding and turning the pulp crush constantly till appearance of juice or hot water extraction methods (Lee W et al, 2006) can be used for production of banana juice. The banana juice attained by these styles is cloudy, thick and slate in color and tends to settle during storehouse. Farther processing similar as enzyme treatment is necessary to produce a ready to drink

clarified banana juice. Pectinase enzyme can be significantly used to reduce the turbidity of the juice which will make the storage of juice very efficient and feasible. The project research work has been performed with following objectives:

- Screening of Microbial sources for pectinase production.
- Production of Pectinase enzyme by Fermentation.
- Application of isolated enzymes.

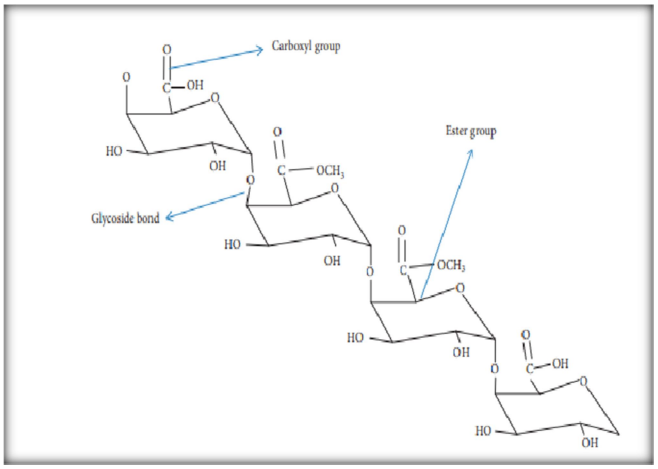


Fig. 1 Pectin Structure

2. Methodology:

i. Experimental material and experimental site: The culture is isolated from soil samples and the banana peels are collected from local areas and used as experimental materials. The experiment is performed in MGM Institute of Biosciences, Aurangabad during 2022-23.

ii. Screening and isolation of pectinolytic organisms.

The organism which are to be used for production of pectinase enzyme are isolated from soil samples using serial dilution method. The microorganisms are grown on nutrient agar medium and incubated at 370C for 24 hrs. The colonies grown are checked for presence of pectinase producing colonies. The colonies are spread on pectin agar plates and kept for incubation at 300 C for 48 hrs. After the incubation 0.3% Congo red solution is added to plates and clear zone is observed. The organism which produces maximum clear zone is selected as organism for pectinase production.

iii. Substrate preparation for enzyme production.

Bananas are collected from local areas. The bananas are washed under tab water and peeled with hand. The peels are then dried at 45 °C for 24 h and then grinded using commercial grinder and passed through 200 mesh sieve to obtain banana peel powder. The powder thus produced is then used as substrate for the production of Pectinase enzyme.

iv. Pectinase Production.

Experiments are carried out in 250 ml Erlenmeyer flasks containing KH2PO4 (0.02 %w/v), MgSO4 (0.01 %w/v), (NH4)2 SO4 (0.04 %w/v), FeSO4 (0.01 %w/v), MnSO4 (0.001 %w/v) in 100 ml distilled water. The concentration of the pectic substrate (Dried banana peel powder) is varied as per the experimental design for optimization. The medium is then sterilized and cooled to room temperature. The pH of the medium is adjusted to 5.8 using citrate buffer. The sterilized flasks are then inoculated with approximately 2× 108 spores of selected organism and kept for incubation in an incubator shaker with a shaking speed of 150 rpm for pre-determined time and temperature as per experimental design. After incubation the culture filtrate is cooled to 4 °C and centrifuged for 10 min at 10,000 rpm in refrigerated centrifuge. Supernatants are collected which are then used for further investigation. Further bulk production of pectinase is carried out at the optimum condition for partial purification and clarification of banana juice.

v. Partial purification.

Partial purification of the crude enzyme is done using ethanol by the method described by Khairnar et al. (2009) with slight modifications 50 ml of the supernatant is treated with three volumes of chilled ethanol and allowed to stand for 15 min at 4 °C as lower temperature will facilitate separation of proteins by reducing the solubility without any damage to the enzyme protein, and centrifuged at 10,000 rpm for 10 min. The precipitate thus obtained is dissolved in 15 ml distilled water and used for further investigation.

vi. Polygalacturonase (PG) activity

Polygalacturonase activity was determined by incubating 0.5 % polygalacturonic acid (Sigma-Aldrich, USA) in 0.2 M citrate buffer (pH 5.8) with the enzyme extract. Reaction was carried out at 37 °C for 60 min. The Nelson-Somogyi method was used determine the release of reducing groups (Somogyi 1952). A calibration curve was made using galacturonic acid (Sigma-Aldrich, USA) as standard. One unit of polygalacturonases activity was defined as the amount of enzyme that released 1 µ mol of galacturonic acid per minute.

vii. Enzyme Activity.

The enzyme activity was tested by DNSA method. The standard curve was plotted by taking pectin as standard. The different concentrations (50-250 mg) of Standard were taken in test tube, 1 ml of DNSA reagent was added in test tubes and the tubes were incubated in water bath for 10 min. Then the volume was make-up to 5ml with distilled water and the absorbance was recorded at 660 nm. The test sample were taken in separate test tube pectin was used as substrate for the enzyme to act.

viii. Juice extraction and clarification.

Ripe bananas are obtained from Local market for juice extraction. The bananas are washed, hand peeled and cut into pieces. Banana pieces are mixed with distilled water in the ratio of 1:2 (w/v) and blended in a commercial blender. The juice is then separated using double fold cheese cloth. For clarification, the extracted juice (15 ml) was incubated for 30 and 60 minutes at 35 °C with varying concentrations of crude and partially purified pectinase.

3. Results:

Screening of Microbes: Clear zones were observed on the pectin plates of *Aspergillus niger* and yeast plates. This indicates the production of pectinase enzyme by the organism.

Sr. No.	Standard Solution	Distilled Water	DNSA Reagent		Distilled Water	Concentration	Absorbance at 660 nm
1.	0	1.0	1 ml	Incubation in water bath for 10 min	3 ml	0	00
2.	0.2	0.8	1 ml		3 ml	50	0.12
3.	0.4	0.6	1 ml		3 ml	100	0.24
4.	0.6	0.4	1 ml		3 ml	150	0.36
5.	0.8	0.2	1 ml		3 ml	200	0.48
6.	1.0	0	1 ml		3 ml	250	0.72

Table 1. DNSA Test for Enzyme Activity

Table 2. Enzyme activity for fungus

Sr. No	Substrate	Distilled Water	Enzyme	Incubation at room Temperature for 10 min	DNSA reagent	Incubation in water bath for 10 min	Absorbance at 660 nm
1	0.1	1.8	0.1		1 ml		0.50
2	0	2.0	0.1		1 ml		0.26

Blank Value: 0.50 – 0.26 = 0.24

Table 3. Enzyme activity for yeast

Sr. No	Substrate	Distilled Water	Enzyme	Incubation at room Temperature for 10 min	DNSA reagent	Incubation in water bath for 10 min	Absorbance at 660 nm
1	0.1	1.8	0.1		1 ml		0.48
2	0	2.0	0.1		1 ml		0.24
0.48 – 0.24 = 0.24							

Polygalacturonase Activity: Pectinase was produced at optimum condition in sufficient amount to carry out further experiments. Some part of the pectinase thus produced was partially purified using ethanol. The polygalacturonase activity and protein content of the partially purified pectinase are presented in Table 4 & 5.

Table 4. PG activity for fungal enzyme

Enzyme	PG activity
Crude enzyme	6.8
Partially purified enzyme	20.9

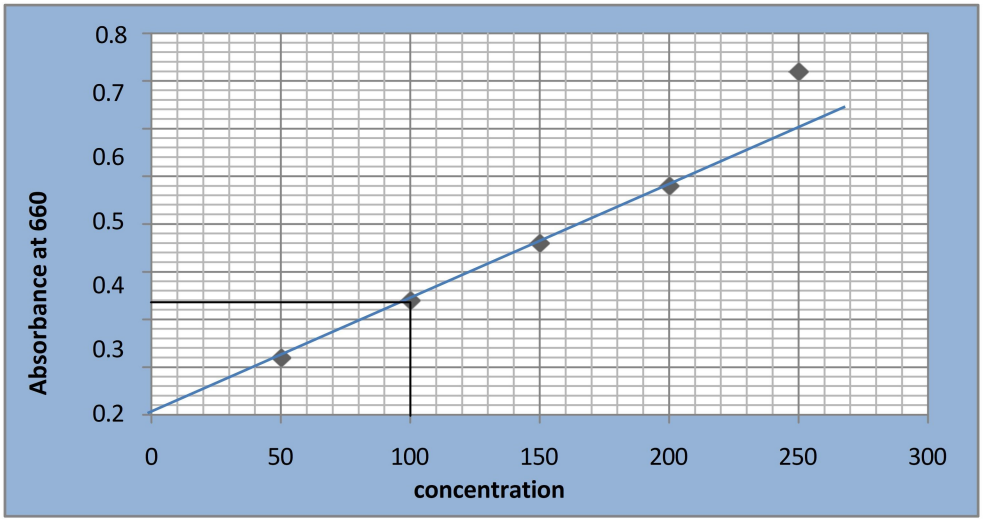
Table 5. PG activity for yeast enzyme

Enzyme	PG activity
Crude enzyme	6.8
Partially purified enzyme	20.9

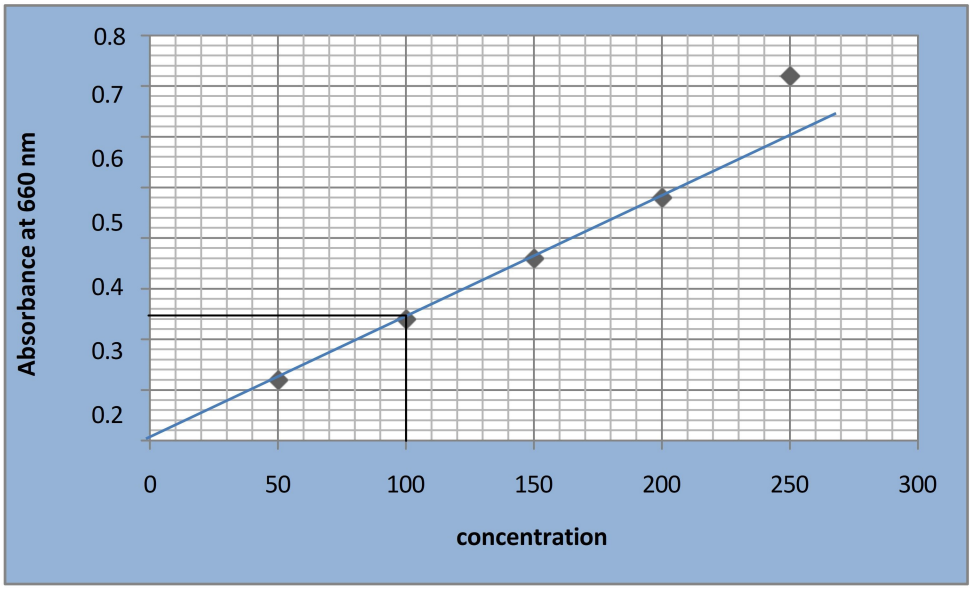
Effect on clarity of banana juice: Significant reduction in the turbidity of juice is seen after the use of Enzyme. Absorbance of the juice was measured at 660 nm before the use of enzyme and it was recorded to be 1.83. After treatment with enzyme and incubation for 60 min the absorbance was measured again.

Table 6. Absorbance of Banana juice after Enzyme treatment

Source	Crude	Partially purified
Yeast enzyme	0.62	0.23
Fungal enzyme	0.59	0.20



Graph 1. Enzyme Activity of fungal enzyme (Enzyme Activity Calculation (fungus): 100 µg/ml/min)



Graph 2. Enzyme Activity of yeast enzyme (Enzyme Activity Calculation (yeast): 100 µg/ml/min)

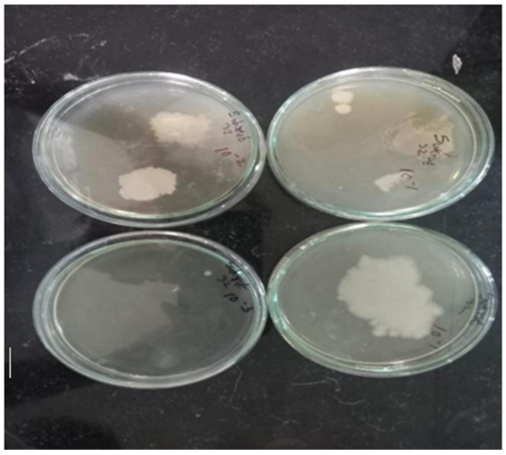


Fig. 1. Serial dilution

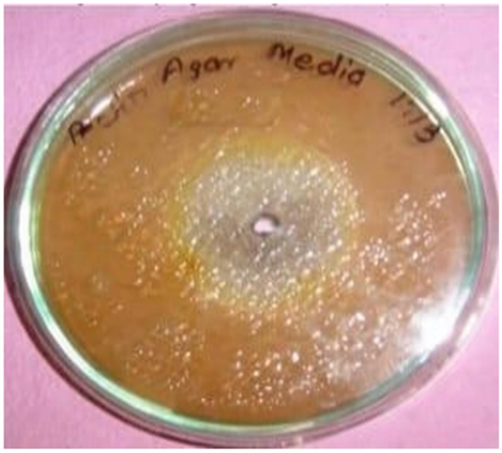


Fig. 3. Pectinase screening (*A. niger*)

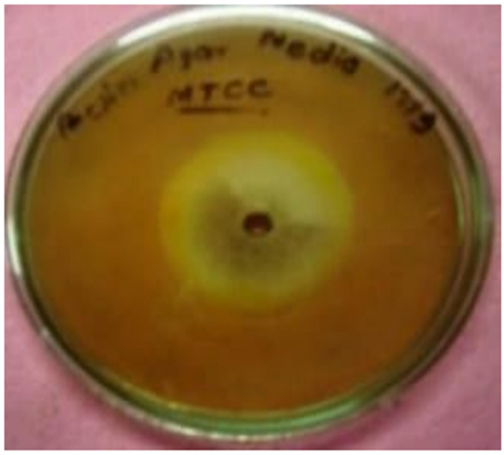


Fig. 4. Pectinase screening (yeast)

Fig. 5 Banana peel powder

Fig. 6. Partially purified enzyme

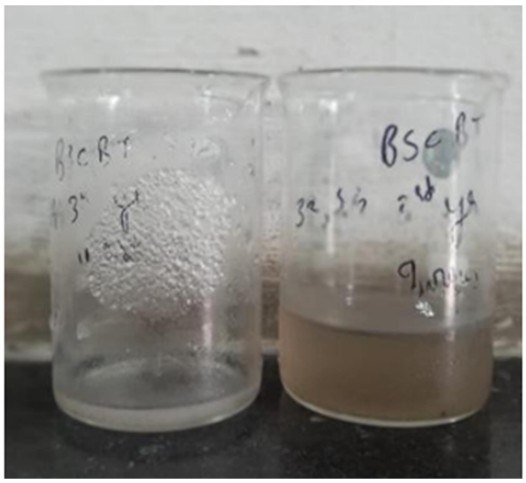


Fig. 7. Crude enzyme

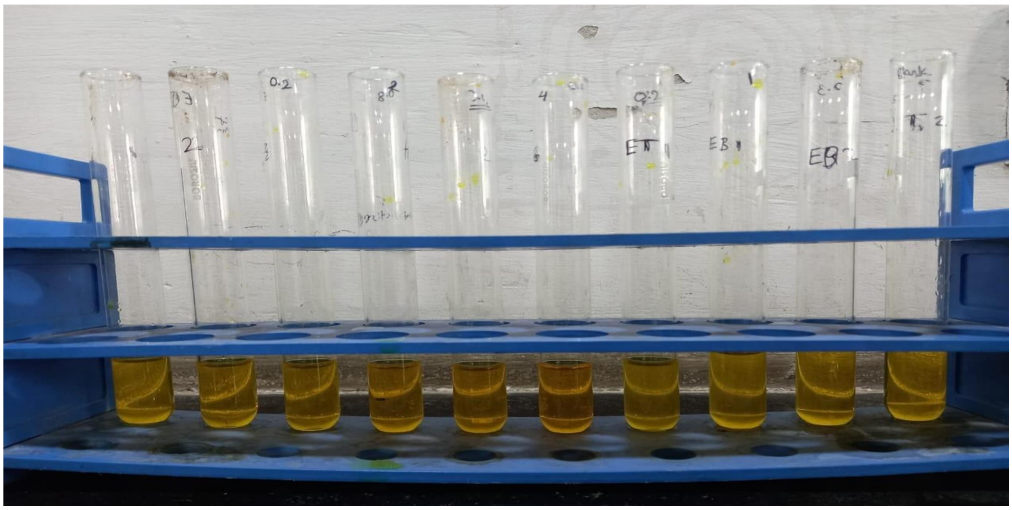


Fig. 8. Enzyme activity (DNSA test)

4. Discussion:

A class of enzymes known as pectinase is categorised based on how they break down pectic substances. Methoxy groups, for instance, are eliminated from highly or partially esterified galacturonan by methylesterases. Exopoly-glacturonases release galacturonic or digalacturunic acid residues from the non-reducing end of homogalacturonan, while endo polygalacturonases randomly catalyse the hydrolysis of glycosidic bonds. Pectinase is essential to the food industry. These enzymes are helpful for extracting fruit juice, clarifying wine, concentration and fermenting tea, cocoa, and coffee, extracting vegetable oil, making jam and jellies, and pickling (Barman S et al. 2015). These enzymes are also employed in the pulp and paper industries, in the bleaching of paper, in the bio-scouring of cotton, in the retting and degumming of plant fibres, in the extraction of oil, in the treatment of wastewater, in the additives for poultry feed, in protoplast fusion technology, and in the synthesis of bioenergy.

Around 25% of the world's food enzyme production is accounted for by pectic enzymes alone, and the majority of pectic enzyme preparations are used in the fruit processing industry (Patil S et al, 2006). Pectinase is produced and secreted by bacteria, fungi, and yeast (Rombouts F et al, 1980). Pectinase has been produced using a variety of fruit and vegetable processing wastes (Bari M et al, 2010; Kumar Y et al, 2012). Bananas are the second most produced fruit globally, after citrus. Although bananas are best consumed raw, they can also be processed to make other goods (Lee W et al. 2006). A cheap and

plentiful agricultural waste residue is banana peel. It is readily accessible in big volumes. It makes up roughly 40% of the fruit's weight when raw, and it's full of protein, carbs, and different vitamins and minerals (Gadge P & Dandu M, 2022). It is an excellent source of nutrients for all living things. In this study, the pectinase enzyme is fermented using banana peel as a raw material. As fermentative organisms, two microbes (a yeast and a fungus) are employed. The enzyme produced is partially purified by ethanol precipitation, and its activity is quantified. Juice clarity on banana juice is another method used to measure the activity of the enzymes.

5. Conclusion:

Peels from bananas are a cheap and plentiful agricultural waste residue. It is readily accessible in big volumes. It makes up about 40% of the fruit's weight when raw and is high in protein, carbs, and a variety of vitamins and minerals. It is an excellent source of nutrients for all living things. Given their affordability, renewable nature, and abundance, banana peels are a promising carbon source and substrate for the production of pectinase. In this study, they are utilised as raw materials for the fermentation of pectinase enzyme, a waste product that was previously used to produce crude pectinase from *A. niger* and yeast. A straightforward method for protein separation called partial purification with ethanol lowers the volume of crude enzyme and increases its concentration, making it more suitable for use in food applications—especially for the clarification of juices. Additionally, partial purification facilitates easy storage of the enzyme. The Polygalacturonases and DNSA tests were used to quantify the generated enzyme's activity. Furthermore, the pectinase enzyme generated in this study demonstrated a demonstrated capacity for juice clarification, suggesting a possible application in the food industry. However, more research is needed to determine the strain's genetic makeup and ensure that it can be used commercially for large-scale food processing and formulation.

References:

Arnau, J., Yaver, D., & Hjort, C. M. (2020). Strategies and challenges for the development of industrial enzymes using fungal cell factories. In *Grand challenges in biology and biotechnology* (pp. 179–210). https://doi.org/10.1007/978-3-030-29541-7_7

Bari, M. R., Alizadeh, M., & Farbeh, F. (2010). Optimizing endopectinase production from date pomace by *Aspergillus niger* PC5 using response surface methodology. *Food and Bioproducts Processing*, *88*(1), 67–72. https://doi.org/10.1016/j.fbp.2009.03.004

Barman, S., Sit, N., Badwaik, L. S., & Deka, S. C. (2014). Pectinase production by *Aspergillus niger* using banana (Musa balbisiana) peel as substrate and its effect on clarification of banana juice. *Journal of Food Science and Technology*. https://doi.org/10.1007/s13197-014-1413-8

Gadge, P., & Dandu, M. (2022). Utilization of Banana and Beetroot waste for Bioethanol Production. *Journal of Emerging Technologies and Innovative Research*, *8*(2), 53–56.

Haile, S., & Ayele, A. (2022). Pectinase from Microorganisms and Its Industrial Applications. *The Scientific World JOURNAL*, *2022*, 1–15. https://doi.org/10.1155/2022/1881305

Haile, S., Masi, C., & Tafesse, M. (2022). Isolation and characterization of pectinase-producing bacteria (Serratia marcescens) from avocado peel waste for juice clarification. *BMC Microbiology*, *22*(1). https://doi.org/10.1186/s12866-022-02536-8

Kc, S., Upadhyaya, J., Joshi, D. R., Lekhak, B., Chaudhary, D. K., Pant, B. R., Bajgai, T. R., Dhital, R., Khanal, S., Koirala, N., & Raghavan, V. (2020). Production, Characterization, and Industrial Application of Pectinase Enzyme Isolated from Fungal Strains. *Fermentation*, *6*(2), 59. https://doi.org/10.3390/fermentation6020059

Khairnar, Y., B, J., Mujapara, A., Jhadav, A., Gupta, M., Gupta, G., Patil, P., Trivedi, S., Gupta, N., Boraste, A., K, V. K., & D, M. (2009). Study of pectinase production in submerged fermentation using different strains of *Aspergillus Niger*. *International Journal of Microbiology Research*, *1*(2), 13–17. https://doi.org/10.9735/0975-5276.1.2.13-17

Kumar, Y. S., Kumar, P. V., & Reddy, O. V. S. (2012). Pectinase Production from Mango Peel Using *Aspergillus* foetidus and its Application in Processing of Mango Juice. *Food Biotechnology*, *26*(2), 107–123. https://doi.org/10.1080/08905436.2012.670830

Lee, W., Yusof, S., Hamid, N., & Baharin, B. (2006). Optimizing conditions for hot water extraction of banana juice using response surface methodology (RSM). *Journal of Food Engineering*, *75*(4), 473–479. https://doi.org/10.1016/j.jfoodeng.2005.04.062

Oumer, O. J., & Abate, D. (2018). Screening and Molecular Identification of Pectinase Producing Microbes from Coffee Pulp. *BioMed Research International*, *2018*, 1–7. https://doi.org/10.1155/2018/2961767

Patil, S. R., & Dayanand, A. (2006). Production of pectinase from deseeded sunflower head by *Aspergillus niger* in submerged and solid-state conditions. *Bioresource Technology*, *97*(16), 2054–2058. https://doi.org/10.1016/j.biortech.2005.09.015

Pires-Cabral, P., Da Fonseca, M., & Ferreira-Dias, S. (2010). Esterification activity and operational stability of Candida rugosa lipase immobilized in polyurethane foams in the production of ethyl butyrate. *Biochemical Engineering Journal*, *48*(2), 246–252. https://doi.org/10.1016/j.bej.2009.10.021

Rombouts, F. M., & Pilnik, W. (1980). Pectic Enzymes. In A. H. Rose (Ed.), *Economic Microbiology, Vol. 5: Microbial Enzymes and Bioconversions* (pp. 227-282). Academic Press.

Somogyi, M. (1952). NOTES ON SUGAR DETERMINATION. *Journal of Biological Chemistry*, *195*(1), 19–23. https://doi.org/10.1016/s0021-9258(19)50870-5

Tabssum, F., & Ali, S. S. (2018). Screening of pectinase producing gram positive bacteria: isolation and characterization. *Punjab University Journal of Zoology*, *33*(1). https://doi.org/10.17582/