International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 10, Issue 9, 2018, pp.-1355-1359.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

ISOLATION, SCREENING AND CHARACTERIZATION OF MICROORGANISMS PRODUCING PECTINASE ENZYME FROM THE *IPOMOEA* SPP. AND ITS POTENTIAL APPLICATION

SHAIKH R., NAYAK J.*, MEHTA M. AND SHAH G.
Department of Biotechnology, Veer Narmad South Gujarat University, Surat, 395 007, Gujarat, India
*Corresponding Author: Email - jitendrajayprakash@gmail.com

Received: August 24, 2018; Revised: September 06, 2018; Accepted: September 07, 2018; Published: September 30, 2018

Abstract- Pectin is a major constituent of primary cell wall of all land plants and surrounded a range of galacturonic acid rich polysaccharides. Pectinase or pectinolytic enzyme, hydrolyze specific substance, those enzymes have a share of 25% in the global sales of food enzymes. Production of this enzyme is influenced by the various aspects such as nature of solid substrate, level of moisture content, presence or absence of carbon, nitrogen, minerals and vitamin supplements. Maximum (optimum) enzyme production is attained when temperature is around 30°C, pH-8.0, pectin as a carbon source, yeast extract as a nitrogen source and incubation time is 72 h. In addition to this, Sweet potato (*Ipomoea* spp.) acts as a better agro substrate and for better production of pectinase. Purified pectinase showed a molecular weight of 45-50 kDa observed by means of SDS-PAGE. Paper chromatography results showed that the presence of isolated pectinolytic enzymes. Pectinolytic enzyme is of significant importance in the application of fruit juice industry in the current biotechnological era. The significance of pectinase enzyme is emerging as a rapid fire, since these enzymes act as a tool in the variety of industries like fruit processing industry, vegetables processing industry, paper industry *etc*., so owing to their vast and variety applications, newer microorganisms are to be screened for the production of pectinase enzyme with desirable properties.

Keywords- Pectinolytic enzyme, Sweet potato (*Ipomoea* spp.), SDS-PAGE, Paper chromatography

Citation: Shaikh R., et al., (2018) Isolation, Screening and Characterization of Microorganisms producing Pectinase Enzyme from the *Ipomoea* spp. and its Potential Application. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 10, Issue 9, pp.-1355-1359.

Introduction

The use and implementation of microorganisms, such as bacteria, yeasts and predominantly fungi, by the food industry sector has led to a high expansion of food industry with pertinent economical assets. Fermentation of alcoholic beverages, dairy products, organic acids and drugs with better remark to the production with those microorganisms; which also incorporates antibiotics are the most crucial examples of microbiological processes. The enzyme industry, as it is contemporarily known, is the outcome of a fast development of biotechnology, particularly during the past four decades. Since prehistoric times, enzymes obtained through nature have been utilized in the production of food products such as cheese, beer, wine and vinegar [1]. Pectin, a heterogeneous polysaccharide, is a major constituent of cereals, vegetables, fruits and fibers, made of α (1-4) D-galacturonic acid residue [2]. Pectinases hydrolyzes pectin present in the specific substrate and finds its use as well as implementation in various industrial processes. These enzymes lead to the breakdown of composite polysaccharides of plant tissues containing pectin into simpler molecules like galacturonic acid. Acidic pectinases helps in reducing the cloudiness and bitterness of fruit juices while alkaline pectinases are utilized in the textile industry for the retting and degumming of fiber crops, production of better quality paper, fermentation based on the substrates like coffee and tea, oil extractions and treatment of pectic waste water [3]. Pectinases, the soluble complex polysaccharides that is present widely in plant cell walls. Pectinase accounts for 10% of worldwide industrial enzymes produced and their market is boosting day by day. Many studies have been directed and regulated on the production of pectinases from various microorganisms. But a few works have been published about cost-effective production of enzymes. The difficulties to obtain the appropriate substrate might be the biggest problem to develop such studies. A suitable substrate should be cost-effective and to provide all necessary nutrients to the microorganism, if not, it would be necessary to supplement them externally. There are different low-cost substrates like Sweet potato; which is used for cost-effective production of the pectinase enzyme. Of the various wastes accumulating in the environment, Sweet potatoes were considered as the most predominant substrate due to its vast usage in starch processing industries and easy availability in India [4]. In this paper we report the production of pectinases by isolated strain of microorganisms from the soil by submerged fermentation using sweet potato as a substrate. Hence, it was attempted to study on its role as substrate for pectinase production. The enzymes produced through microbial origin were found to be more advantageous and beneficial than others. In the present study production of pectinase using Sweet potato was carried out and the production conditions were optimized.

Materials and Methods

Isolation of Microorganisms

Soil sample was collected from the botanical garden near Department of Biotechnology, V.N.S.G. University, Surat, India; 1 g was weighed and dissolved in 100 mL distilled water. With the help of pipette, 0.1 mL was spread on pectin agar plate (Pectin- 0.75g, Agar- 4.5g, $CaCl_2$- 0.12g, Sodium lauryl sulfate- 0.02g, $NaNO_3$- 0.4g, Tryptone- 0.2g, Crystal violet- 2 drops, pH- 7.2 ± 0.2). Then, plates were incubated at 30ºC. Colonies were observed on pectin agar plates at regular interval. Plate assay technique were performed for the detection of bacteria that degrades pectin by streaking an inoculum of identified bacterial strain in pectin agar plate. Further plates were incubated for 48 h at 30ºC. Colonial growth showing the zone formation were observed which indicates the degradation of pectin. Organisms were identified by morphological identification with the help of staining.

Preparation of Inoculum for Bacteria

Luria Bertani medium was supplemented with 1% pectin (1 g pectin/ 100 mL) and

isolated colony was suspended in the prepared media. Flask were incubated in incubator at 37ºC at 200 rpm for 48 h. For enzyme production the fresh overnight culture was used.

In vitro Characterisation Of Pectinase Activity

Effect of substrate on enzyme activity

Crude substrate was prepared; volume of 100 mL in 250 mL Erlenmeyer flask. From the prepared overnight fresh culture, 5% inoculum of bacterial culture was inoculated to the crude substrate in aseptic condition. Flask were incubated for 72 h at 37ºC. After the incubation, the samples were centrifuged at 10000 rpm for 10 minutes. Obtained supernatant was used as a crude substrate for the determination of enzyme activity.

Effect of pH on enzyme activity

Crude substrate (Dried and powdered form of Sweet potato) was prepared; volume of 100 mL in 250 mL Erlenmeyer flask. The adjustment of pH was done in between the range of pH 6 to 8. From the prepared overnight fresh culture, 5% inoculum of bacterial culture was inoculated to the crude substrate in aseptic condition. Flask were incubated for 72 h at 37ºC. After the incubation, the samples were centrifuged at 10000 rpm for 10 minutes. Obtained supernatant was used as a crude substrate for the determination of enzyme activity.

Effect of temperature on enzyme activity

Crude substrate was prepared; volume of 100 mL in 250 mL Erlenmeyer flask. The adjustment of pH was done in between the range of pH 6 to 8. From the prepared overnight fresh culture, 5% inoculum of bacterial culture was inoculated to the crude substrate in aseptic condition. Flask were incubated for 72 h at 37ºC. After the incubation, the samples were centrifuged at 10000 rpm for 10 min. Obtained supernatant was used as a crude substrate for the determination of enzyme activity.

Determination of Extracellular Enzyme Activity

Nutrient agar plate with 1% pectin

The two isolates labelled R1 and R2 were streaked in the nutrient agar plate (Pectin- 1.5 g, Peptone- 0.75 g, Beef extract- 0.45 g, NaCl- 0.75 g, D.W.- 150 mL, Agar- 2.25 g, pH- 7.4 ± 0.2) with 1% pectin. Plates were incubated in the incubator for 48 h at 37ºC. Plates were flooded with 1% Congo red (Congo red- 1 g, D.W.- 100 mL) and the zone of utilization of pectin was observed.

Mc Beth's agar plate

The two isolates labelled R1 and R2 were streaked in the Mc. Beth's agar plate (K_2HPO_4- 0.1g, $MgSO_4.7H_2O$- 0.1g, Na_2SO_4- 0.2g, $(NH_4)_2SO_4$- 0.2g, $CaCO_3$- 0.2 g, Pectin- 0.5g, Agar- 3g, D.W.- 100 mL). Plates were incubated in the incubator for 48 h at 37ºC. Zone of utilization showing white colour surrounded the colony was observed.

Determination of Enzyme Concentration by Bradford Method

Culture filtrate of 1 mL was mixed with 5 mL of Bradford reagent in the test tubes. Absorbance was measured at 595 nm in spectrophotometer. Protein concentration was determined using the BSA (Bovine Serum Albumin) standard.

Partial Purification of The Enzyme

Culture filtrate was transferred in 80% w/v of Ammonium sulphate, precipitation of protein was observed. Precipitated protein was collected by centrifugation at 10000 rpm for 10 minutes. Supernatant was discarded and the pellet was dissolved in 2 mL of 0.5 mM Tris-HCl (pH-8).

SDS-PAGE Analysis of Pectinase

Protein profile was confirmed and analysed by SDS-PAGE. SDS-PAGE electrophoresis was performed in 10% (w/v) gels and samples were heated for 10 min at 45 °C in the sample buffer before loading on wells.

Separation of Enzymes by Paper Chromatography

Mixture of 10 cm^3 of 2% ammonia solution with 20 cm^3 of Propan-2-ol in a clean, 500 cm^3 beaker was prepared, and covered tightly with a piece of aluminium foil. This is to be used as a solvent (Hazard warning: Propan-2-ol is flammable). Clean sheet of chromatography paper with size about 10 cm x 30 cm was placed, light pencil line was drawn to the bottom and about 1.5cm away as shown in figure. Along this line, seven light dots (·) were marked at intervals of about 1.5 cm. A small amount of each appropriate enzyme solution was placed by using capillary tubes on its marked positions along the line on the chromatography paper. The spot on the paper larger than about 3mm in diameter is to be avoided as shown in [Fig-1]. The paper was allowed to dry for a few minutes in air. After drying, the paper was hung from a steel rod kept at the top of a cylindrical glass chamber containing solvent. Make sure that the paper does not touch the wall of the beaker. The solvent rise up was allowed to the paper for at least 2 h. If the time is shorter, the components may not be sufficiently separated for easy identification. The paper was removed and placed it upside down on the desk top to dry. A solution of ninhydrin (2%) was sprayed on the paper lightly but completely, and kept the paper in the fume cupboard until the spray solution is dry. The paper was placed in an oven at 100-110 ºC for about 10minutes, or until all the spots have developed. The spots were marked and the distance of the each spots travelled were measured, also measurement of the distance travelled by the solvent at each position was done and the R_f values for each samples were calculated.

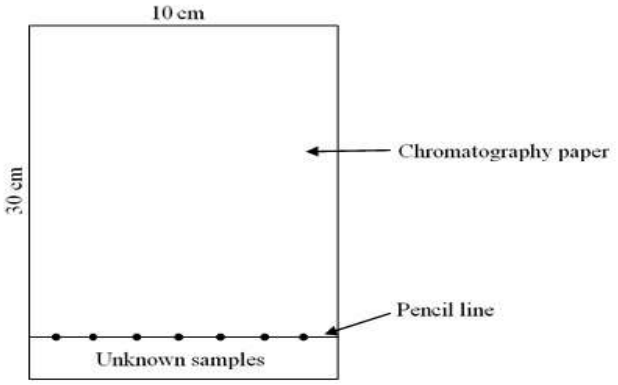


Fig-1 Chromatography paper showing placed sample

Application of Pectinase Enzyme in Fruit Juice Clarification

Two experimental extracts obtained with samples R1 and R2 were used in substrate process for clarification of the fruit juice. The solution was prepared; 10 mL of orange juice and to that 1 mL of enzyme extract was added. For clarification assays, the test tubes of containing mixture of orange juice and enzyme extract were placed to the thermostatic waterbath at 50°C for a reaction time 60 minutes. Subsequently the samples were placed for cooling in an ice bath to interrupt the reaction. The samples were centrifuged for 10 minutes at 10,000 rpm and 20ºC. With the help of whatman paper no. 1, the samples were filtered. The turbidity of samples with control was compared.

Results and Discussion

Isolation of Microorganisms

The serially diluted soil samples were screened for pectinase producing bacteria. Out of several bacteria grown on screening medium, only 3 strains showed clear zones and found as pectinase producers. The strains were then purified using repeated subculture using Nutrient Agar plates and stock culture was maintained in Nutrient Agar slants. Identity and phylogeny of the isolate was analysed through gram staining. The bacterial colony with maximum zone diameter was selected as the best strain and preceded for further studies. From the soil as a source, 2 isolates were isolated as shown in [Fig-2], which were capable of producing pectinase enzyme; and named as R1 and R2. Those isolates were screened for efficient producer using pectin agar plates and subcultured as shown in [Fig-3].

For screening, after the incubation of plates containing different isolates for 24 h, the zone of utilization of pectin were visualized and gram staining was performed; the result is shown in [Fig-2].

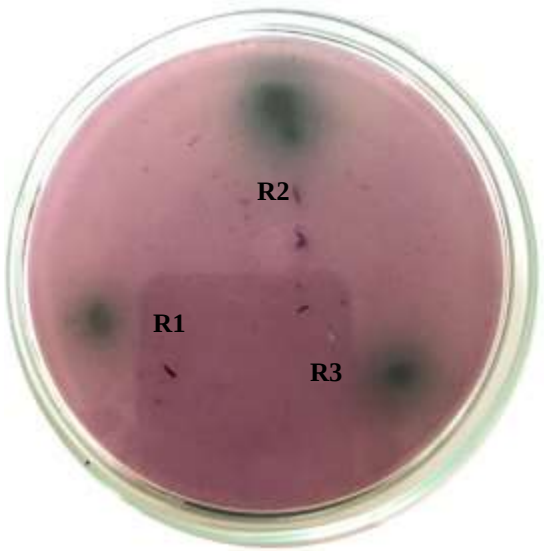
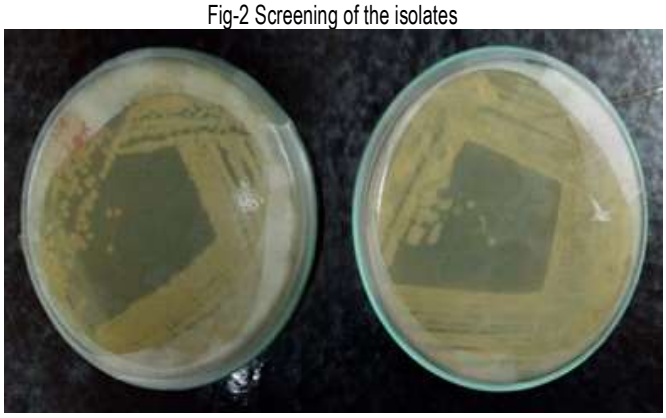


Fig-2 Screening of the isolates



R1 R2

Fig-3 Sub-culturing of the isolates for identification

***In Vitro* Characterisation of Pectinase Activity**

The best strain was isolated and studied in detail on the basis of various parameters for the enzyme production with respect to development of low-cost and easy available medium ingredient. The culture was further screened for pectinase production by submerged fermentation using Sweet potato as substrate. Studies based on production and secretion of pectinase from the microorganisms are important so that to develop enzyme systems which could be directly used for converting biomass into enzymes.

Effect of substrate on enzyme activity

The enzyme activity with the inoculation of the isolates R1 and R2 for the effect of substrate, under the influence of different pH values and its respective data as mentioned in [Table-1], indicated that the enzyme exhibited minimum relative activity with the isolate R1 with respect to R2 as shown in [Fig-4].

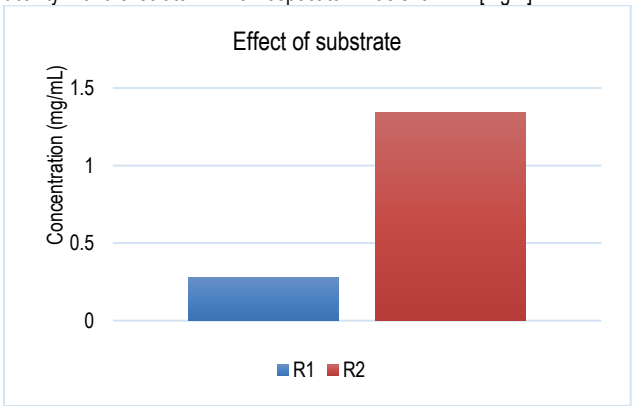


Fig-4 Graph based on Effect of Substrate

Effect of pH on enzyme activity

From our study the enzyme activity with the inoculation of the isolates R1 and R2 for the effect of pH, it was found that the isolates has the potential to produce maximum amount of pectinase at pH-8 and minimum amount at pH-6 as its respective data are mentioned in [Table-1]. This indicates that the optimum pH for better production, pH around 8.0 is optimum for enhanced pectinase production as shown in Figure-5.

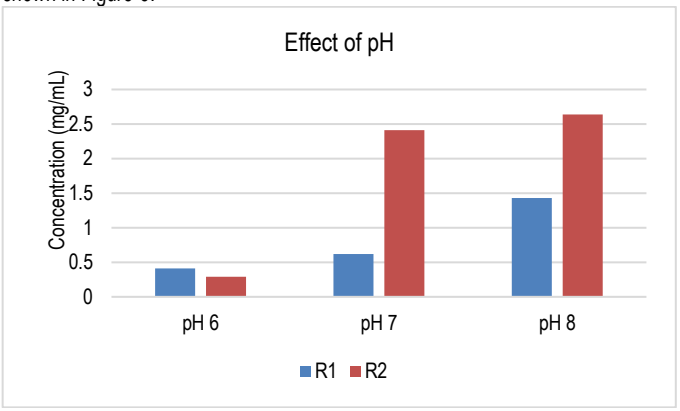


Fig-5 Graph based on Effect of pH

Effect of temperature on enzyme activity

From our study the enzyme activity with the inoculation of the isolates R1 and R2 for the effect of temperature, it was found that the isolates has the potential to produce maximum amount of pectinase at 30°C and minimum amount at 40°C as its respective data are mentioned in [Table-1]. This indicates that the optimum temperature for better production of bacterial isolates is 30°C as shown in figure-6.

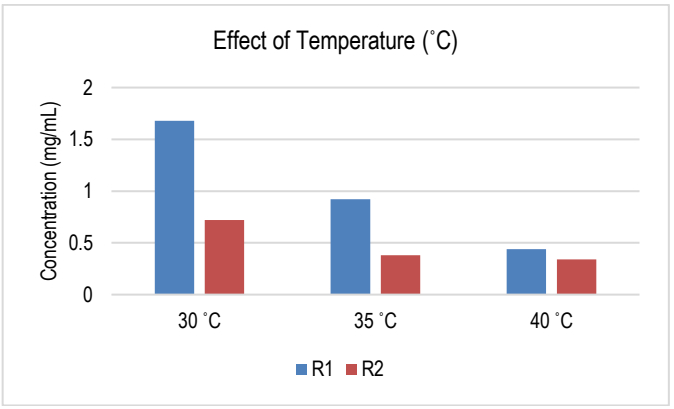


Fig-6 Graph based on Effect of Temperature

Determination of Extracellular Enzyme Activity

Nutrient agar plate with 1% pectin

The zone of utilization can be visualized, which indicates that the pectin is been utilized as a sole source as shown in [Fig-7].

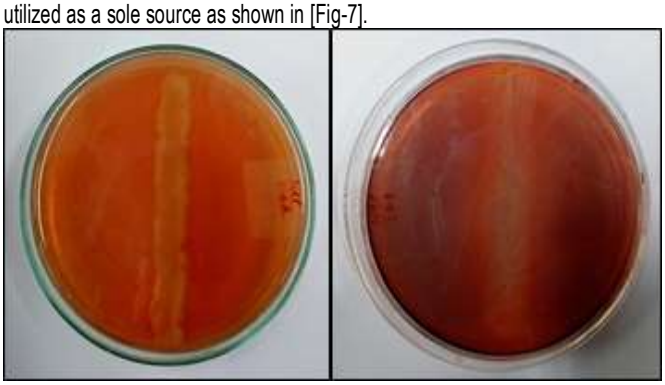


Fig-7 Zone of utilization through isolates R1 and R2

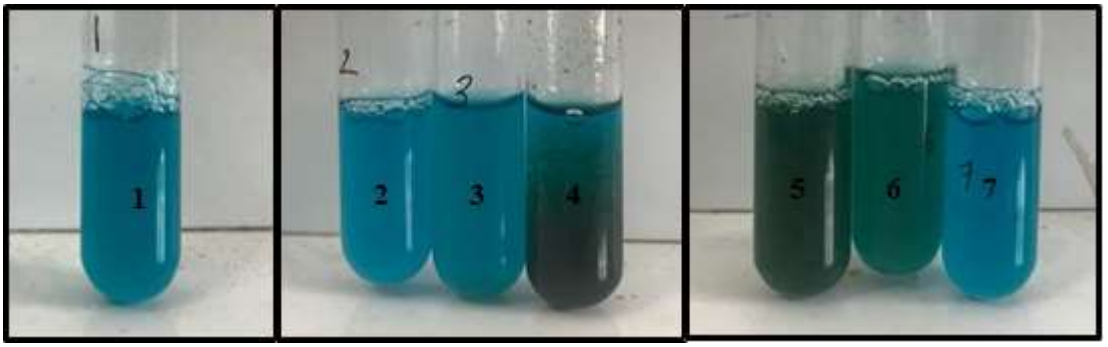


Fig-9 Bradford assay of different enzymes from isolates R1

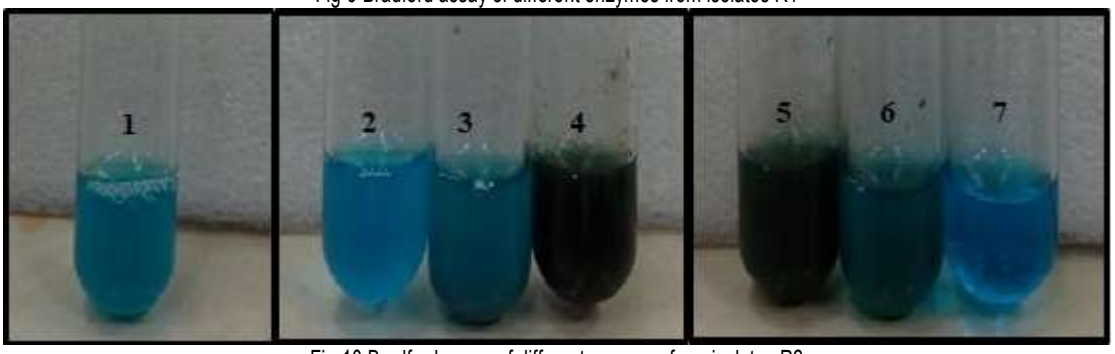


Fig-10 Bradford assay of different enzymes from isolates R2

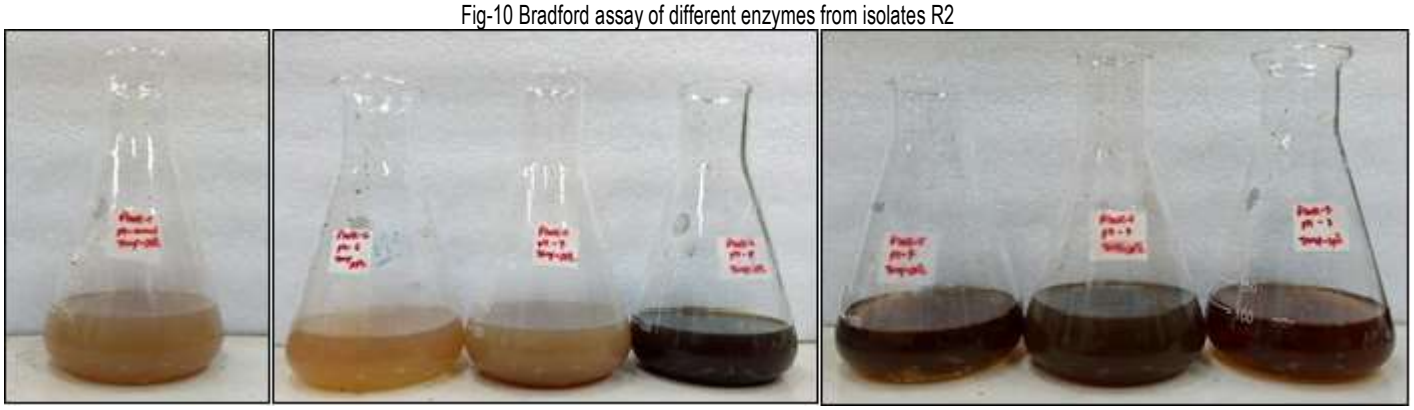


Fig-12 Partial purification of the enzymes

Table-1 Absorbance and Concentration of Samples R1 and R2

Parameters		Absorbance of samples (R1) at 595 nm	Absorbance of samples (R2) at 595 nm	Concentration (mg/mL)	
				Conc. of samples (R1)	Conc. of samples (R2)
Substrate		0.111	1.119	0.28	1.34
	6	0.23	0.115	0.41	0.29
pH	7	0.433	2.15	0.62	2.41
	8	1.211	2.368	1.43	2.64
	30	0.534	1.451	0.72	1.68
Temperature (°C)	35	0.209	0.721	0.38	0.92
	40	0.164	0.259	0.34	0.44

Mc Beth's agar plate

The zone of utilization can be visualized through white zone surrounding the colony, which indicates that the pectin is been utilized as shown in [Fig-8].

Fig-8 Zone of utilization through isolates R1 and R2

Determination of Enzyme Concentration by Bradford Method

With the help of standard BSA as shown in [Fig-11], enzyme concentration of the unknown samples can be measured. This Bradford protein assay is based on the proportional binding of the dye Coomassie to enzymes, which can be clearly seen in the colour variation as shown in [Fig-9]and [Fig-10].

Partial Purification of The Enzyme

The pectinase from both the organisms was purified to homogeneity using various steps. Initially, the enzyme was partially purified by addition of solid ammonium sulphate to the cell-free supernatant as shown in [Fig-12].

SDS-PAGE analysis

The protein profile was analyzed by SDS-PAGE showed the presence of protein bands, around molecular weight 45-50 KDa, which confirms the presence of pectinase enzyme as shown in [Fig-13]. This result resembled with the previous work [5].

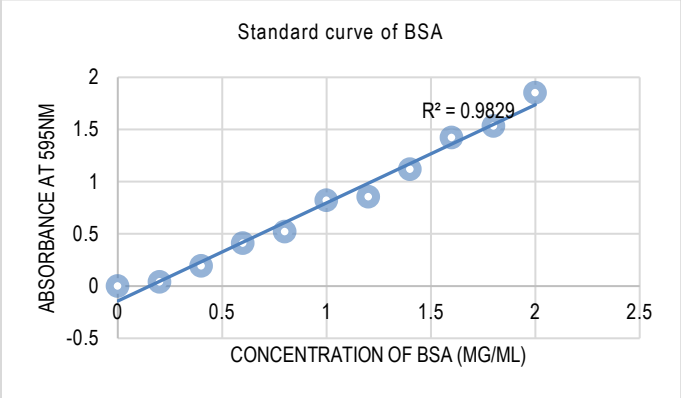


Fig-11 Standard graph of BSA

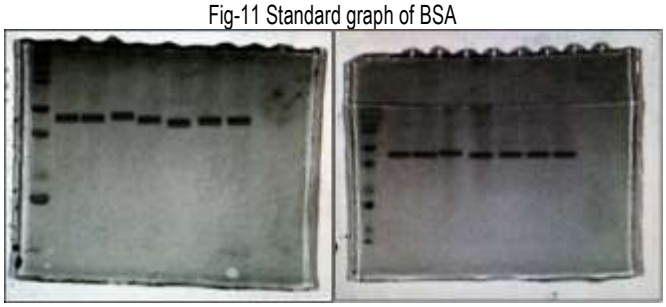


Fig-13 SDS-PAGE analysis of pectinase enzyme from the isolates R1 and R2

Separation of Enzymes by Paper Chromatography

Paper chromatography results shows that the isolated enzymes are similar due to which a straight horizontal line of band can be visualized in the chromatography paper as shown in [Fig-14]. The estimated value of Retention factor values (R_f) is 0.453. ***Calculation:***

Here, the distance moved by the sample containing pectinase enzymes is 4.3 cm and the distance moved by the solvent front is 9.5 cm.

$$Rf = \frac{Distance\ moved\ by\ the\ enzymes}{Distance\ moved\ by\ solvent\ front} = \frac{4.3}{9.5} = 0.453$$

Fig-14 Paper chromatography of the enzymes from isolates R1 and R2

Application of Pectinase Enzyme in Fruit Juice Clarification

Pectinase from isolates R1 and R2 in orange juice shows pectinolytic activities by clarifying it as compared to control tube as shown in [Fig-15]

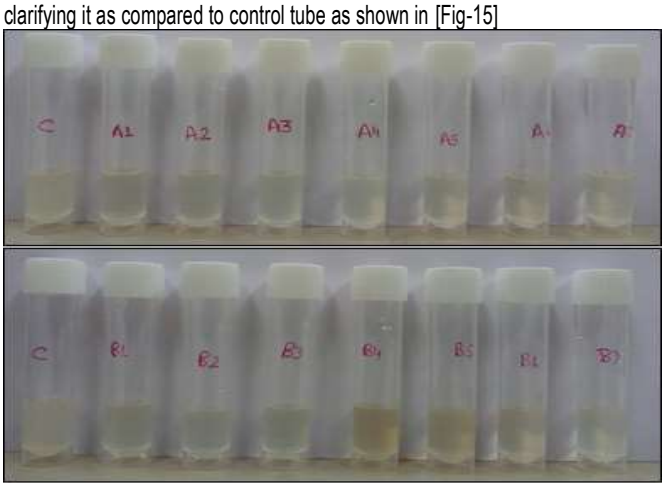


Fig-15 Fruit juice clarification through the enzymes isolated from isolates R1 & R2

Conclusion

The pectinase enzyme producing bacterial strain was isolated from the *Ipomoea* sp. (Sweet potato). The isolated bacterial strain was identified as *Pantoea* sp. and *Sphingomonas paucimobilis*. The production and optimization studies revealed that isolated strains requires 30°C, pH 8.0, pectin, yeast extract, and 72 h of incubation time for higher pectinase enzyme production. The partial purification of pectinase was carried out by ammonium sulphate precipitation. The partially purified pectinase was characterized and the protein profile was analyzed in SDS-PAGE by showing the suitable bands. The current study clearly shows and describes that the microbes can be used in fruit juice manufacturing and in various industry for cost effective production of pectinase. Another major advantage is that the enzyme's properties can be altered by altering the genes responsible for the particular characteristics through rDNA technology for enhanced production.

Application of research: The extracted enzymes has a potential application in fruit juice clarification.

Research Category: Enzyme Technology

Abbreviations: SDS-PAGE: Sodium Dodecyl Sulfate- PolyAcrylamide Gel Electrophoresis, BSA: Bovine Serum Albumin

Acknowledgement / Funding: The authors are thankful to the Head of the Department, Department of Biotechnology, V.N.S.G. University, Surat-395007; for giving research facilities and encouragement.

***Research Guide: Dr. Gaurav Shah**
University: Veer Narmad South Gujarat University, Surat, 395007, India
Research project name: M. Sc. Biotechnology Thesis

Author Contributions: All author equally contributed

Author statement: All authors read, reviewed, agree and approved the final manuscript

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Sample Collection: Botanical garden near Department of Biotechnology, V.N.S.G. University, Surat, India

References

[1] Kirk O., Borchert, T. V., Fuglsang, C. (2002) *Current Opinion Biotechnology, Vol.* 13, 345-351

[2] Alkorta I., Garbisu C., Llama M. J., and Serra J. L. (1998) *Industrial applications of pectic enzymes: A review, Process Biochemistry*, 33, 21-28.

[3] Solbak A.I., Richardson T.H., McCann R.T., Kline K.A., Bartnek F., Tomlinson G., Tan X., Parra-Gessert L., Frey G. J., Podar M., Luginbuhl P., Gray, K. A., Mathur, E.J., Robertson, D. E., Burk, M. J., Hazlewood, G. P., Short J. M., and Kerovuo J. (2005) *Journal of Biological Chemistry, 10,* 9431-9438.

[4] Manas R.S., Shaktimay K., Ramesh C.R. (2009) *Braz. J. Microbiol*., 40, pp-636–648.

[5] Belarbi A., Hachet C., Helfer A.C., Gognies S., Gainvors (2000) *J Biotechnology*, 24, pp-296-300.