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Isolation, Screening and Industrial Applications of Pectinase Isolated from Kalaburagi Region

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Abstract: ***Among many industrial enzymes pectinase plays an important role in many different types of industries like beverage industry and waste water treatment. In the present investigation, a total of 10 different strains are isolated from 5 different soil samples from different regions of vegetables and fruit dump yards in Kalaburagi region. Among 10 strains 5 strains showed maximum pectinase activity among which GSH-5 showed highest pectinase activity of 70U/mL which was further selected fro optimization by physical and chemical parameters by increasing the activity to 120U/mL further the purified enzyme was subjected to clarification of the juice. It was observed that fruit juice has got clarified within 3-4 hours. It was indicated that the bacterial pectinase was effective in the clarification of fruit juices. From this enzyme was found to the prominent candidate for commercial application.***

Keywords: Pectinase, Beverage industry, Kalaburagi, Juice Clarification

1. Introduction

Complex polysaccharides known as pectic compounds consist of simpler molecules, including galacturonic acids. Pectin is found in the middle lamella and cell wall of higher plants and is broken down by a group of enzymes referred to as pectinase. (Ramachandran Sandhya and Kurup G. 2013).

Pectic substances are categorized based on their mechanisms of action. For instance, methyl esterase eliminates methoxy groups from highly or partially esterified galacturonan. Polygalacturonases facilitate the hydrolysis of glycosidic bonds either randomly (as in endopolygalacturonase) or from the nonreducing end of homogalacturonan, resulting in the release of galacturonic residues (as seen in exopolygalacturonases). Despite possessing anti-cholesterol and various health benefits, humans are unable to consume it directly due to its polysaccharide composition. To address this, enzymes are employed to decompose pectin into simpler sugars while preserving its inherent properties.

Most of the industrial demand for enzymes is originated from microorganisms. Due to their high growing capability, short life span, and easiness of genetic manipulation, microorganisms are preferred in industry for enzyme production. Microbial enzymes are supplied, well-standardized, and marketed by a few competing companies. Among these industrially important enzymes, pectinases have a special significance due to their multiple uses in important sectors such as food, textile, beverages, pulp and paper, and biofuel industrial. Microbial pectinases account for twenty five percent of the worldwide food and industrial enzyme scale and market increase from time to time (KC, S et al.,2020).

Pectinase (pectinolytic enzymes) consist of a mixture of complex enzymes that catalyze specifically the hydrolysis of pectin including substrate. These enzymes divide into three main classes that catalyze depolarization, demethylation, & de-esterification reactions. Polygalacturonase that catalyze the breakdown of ALFA 1-4 glycosidic bonds between two galacturonic acid residues in the smooth region of the pectin molecule is a depolymerizing enzyme (Hatice Aysun Mercimek Takc and Filiz Ucan Turkmen 2016).

These pectinases have wide applications in fruit juice industry and wine industry. In fruit juice industry, it is used for clarification, where reduction in viscosity is caused which ultimately leads to formation of clear juice. They increase the yield of juices by enzymatic liquefaction of pulps; these pectinases also help in formation of pulpy products by macerating the organized tissue into suspension of intact cells. In wine industry, pectinases are mainly used for decreasing astringency by solubilising anthocyanins without leaching out procyonid polyphenols, and pectinases also increase pigmentation by extracting more anthocyanins

Commonly used bacteria & fungi for the production of pectinases are species Aspergillus, Penicillium, Rhizopus, Bacillus substillis. Pectinases can be produced by 2 types of fermentation i.e., Solid state & submerged fermentation. Solid state fermentation has a number of advantages over submerged fermentation. Pectinases play vital role in the food industry, oil extraction, textile industry, coffee & tea fermentation etc. Acidic pectinases clarify juices at significant levels. They increase the yield of juice bye pulp liquefaction (Yahya s Al Qahtani 2022).

2. Material and Methods

Collection of Samples:
Soil sample were collected from place in farm lands, dump yard of fruits wastes near Gulbarga central and surrounding regions of Kalaburagi. The sample was labeled after collected.

Enrichment of soil sample:
Dissolving 10 grams of each sample in 100ml of distilled water and filtering it using a sieve was carried out. Separately, 100ml of sterile nutrient broth was inoculated at 37^0C and 180 rpm in rotary shaker incubator. The sterile enrichment broth (Pectin 0.5gm, yeast extract 0.1gm, potassium di phosphate) was used to inoculate 5ml of each overnight culture in it and were subsequently incubated at 37^0C and 180 rpm in rotary

shaker incubator for 48 hours. (AjitKumar and Rita Sharma 2012).

Screening of Pectinase Producing Bacteria:
The strain obtained above was subjected to screening for pectinase production, as explained by Yahya s. Al Qahtani et al. 2022. The bacterial culture screened qualitatively for pectinase production by plate method on pectin agar medium (PAM) containing (g/l); Ammonium sulphate;2, Yeast extract; 1, Disodium phosphate;6, Potassium dihydrogen phosphate; 3, Pectin ;5, Agar; 20, in 1000ml of distilled water inside conical flask. The pH of the medium was adjusted to 7.0± 0.2 using digital Ph meter. The media was sterilized at temperature of 121^0 c for 15 minutes in autoclave. Pectinase production was indicated by a clear halo zone around the colonies after the Petri plate were flooded with iodine solution. (HaticeAysunMercimek and FilizUcan Turkmen 2016).

Production or Enzyme assay:
The production medium at pH 7.0 was the same as the one used for the isolation and screening but with no agar on it. The bacterium with the highest clear zone diameter was considered with 1% inoculum. The inoculated fermentation medium was incubated at 37 °C on a shaking incubator for 48 h. After incubation, the sample was centrifuged at 10,000 rpm for 5 min at 4 °C. The supernatant was collected and used as a crude enzyme. respectively, with pectin substrate and glucose as standards. The enzyme activity (U) was defined as the amount of enzyme that produced 1 µmol of product per min per ml under the assay conditions. Pectinase activity was determinations using 3,5-Dinitrosalicylic acid (DNS)Method at 540 nm, using UV visible spectrophotometer. The observation was recorded at 24 hours, 48 hours, 72 hours, 96 hrs. (Yahya s Al Qahtani and Sunil s, 2022).

$$\text{Relative activity} = \frac{\text{Activity of sample (U)} \times 100}{\text{Maximum enzyme activity (U)}}$$

Biochemical Test:
To identify the screened isolates, the biochemical properties of the bacterial isolates with high pectinase production ability were studied by different biochemical test. Indole test, Methyl red test, Voges Proskauer test, Citrate test, Urease test, Starch hydrolysis.

Physical and Chemical Optimization:
Factor like Temperature, incubation time, pH and nitrogen source were optimized during experimentation for maximizing pectinase activity of the isolates. Optimization studies included various incubation time (24hours, 48 hours, 72 hours, 96 hours,). Temperature (30ºC ,35ºC ,40ºC, 45ºC) and pH value adjusted initially using HCL or NaCl solutions (6.0, 6.5, 7.0, 7.5, 8.0) nitrogen sources used are beef extract, yeast extract, peptone, (0.1gm respectively) (Saratale R.G et al 2014).

Application of the Pectinase Enzyme in Fruit Juice Clarification:
Fruit juices are turbid and cloudy after their extraction from pulp. This un-clarified juice can be clarified using pectinase enzyme under its optimized conditions. To observe the effect of crude pectinase enzyme on fruit juice clarification, two test were labelled as test and control. 10ml of orange and pineapple juice and 1ml of crude pectinase enzyme was taken into the test tube labelled as test while the other test tube containing juices was kept as control without adding enzymes. The contents of the tubes were agitated to mix the enzymes throughout the juice. The experiment was kept for 3-4 hours and the results were recorded,

3. Results

Isolation of Pectin Producing Micro-Organisms:
Pectin hydrolyzing bacteria were isolated from the collected soil samples from different regions of Kalaburagi using serial dilution technique by Pectin Agar Mediaproposed by Hatice Aysun Mercimek and Filiz Ucan Turkmen 2016, supplementing with pectin 0.5%.

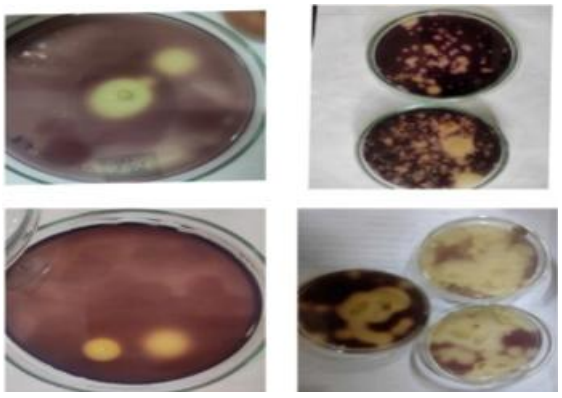

Figure 1: Isolation of pectin hydrolyzing bacteria from different soil samples

Selection of Potent Pectin Producing Micro-organisms:
The formation of hollow clear zone around the colonies when flooded with iodine solution (0.1N), indicates the positive results for pectin hydrolyzing bacteria. Among 5 isolate I have selected five high zone showing bacterial strains and were screened out by incubating them in 100ml of PAM media. The pH was adjusted to 6.5. The plates were incubated at 37^0C for 24-48hrs. The plates were flooded with iodine at every 24 hours interval for formation zone

Table 1: Zone of Clearance of different strains

S. No	Strains	Zone of Clearance(mm)
1	GSH-1	10mm
2	**GSH-2**	**32mm**
3	GSH-3	14mm
4	GSH-4	18mm
5	GSH-5	8mm

Pectinase Assay:
Pectin hydrolysis activity was expressed in terms of % hydrolysis and was determined by maintaining the decrease in acceleration at absorption maximum of pectin using UV visible spectrophotometer at 540nm using 3,5-Dinitrosalicylic acid (DNS)

Method
The observation was recorded at 24 hours, 48 hours, 72 hours, and 96 hours respectively (Yahya s Al Qahtani and Sunil S, 2022).

$$\text{Relative activity} = \frac{\text{Activity of sample (U)} \times 100}{\text{Maximum enzyme activity (U)}}$$

Table 2: Pectinase activity

S. No	Strains	Activity
1	GSH-1	40 U/mL
2	**GSH-2**	**70 U/mL**
3	GSH-3	48 U/mL
4	GSH-4	32 U/mL
5	GSH-5	28 U/mL

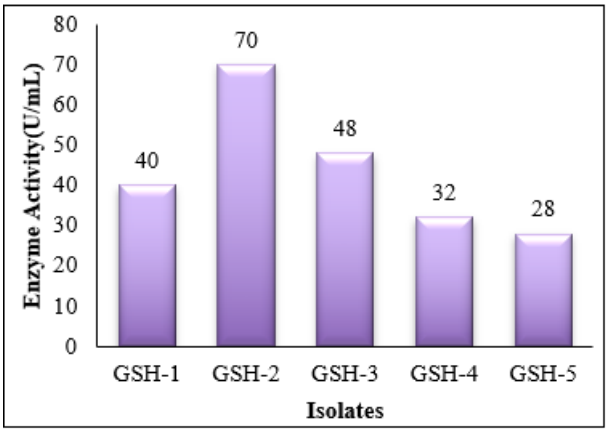

Figure 2: Graph showing Activity of Isolates

Characterization of Isolate of GSH-2:

The isolate GSH-2 was subjected to Gram staining to identify the obtained strain as Gram positive bacteria and Gram-negative bacteria. Bacterial cells isolated showed as gram-positive bacteria. Cells were rod-shaped, non-motile and non-spore forming. Growth occurs at a temperature of 30-45^{0}C and pH 6.0. The optimal growth temperature and pH are 37^{0}C and pH 6.5 respectively. Further isolate was subjected to Biochemical analysis and the obtained results are tabulated in below table.

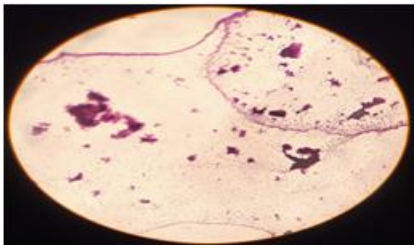

Figure 3: Gram staining of isolates GSH-2

Table 2: Biochemical test of isolate GSH-2

Sl. No	Biochemical Test	Reaction
1	Indole test	Positive
2	Methyl red test	Positive
3	Voges Proskauer test	Negative
4	Urease test	Positive
5	Starch hydrolysis test	Negative
6	Citrate test	Positive

Optimization of isolate GSH-2:

Effect of different parameters like (Temperature, pH, Carbon source and Nitrogen sources) were studied to find its influence on the production of pectinase in the basal media. Finally enhancing the pectinase activity from 70U/mL to 120U/mL

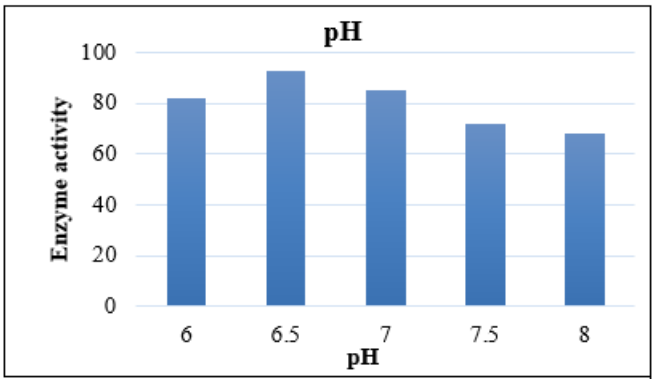

Figure 4: Effect of pH

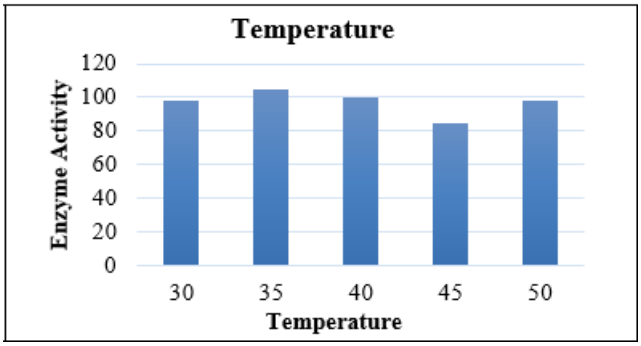

Figure 5: Effect of Temperature

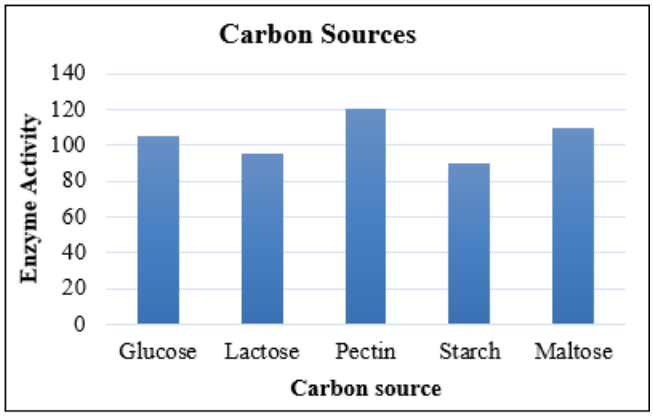

Figure 6: Effect of Carbonsource

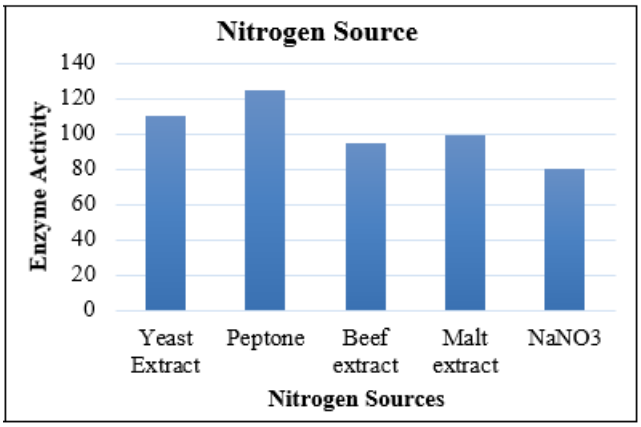

Figure 7: Effect of Nitrogen source

Clarification of Juice:

The isolate GSH-2 was subjected to Juice clarification. Fresh orange and pine apple juice were selected for the present procedure. The purified pectinase obtained from isolate GSH-2 was added to un-clarified juice of 10 ml orange and pine apple juice was taken in different test tube. About 1ml of pectinase enzyme was added the test tubes. It was observed that fruit juice has got clarified within 5-6 hours. From the obtained results it was found to be effective in clarification of juice.

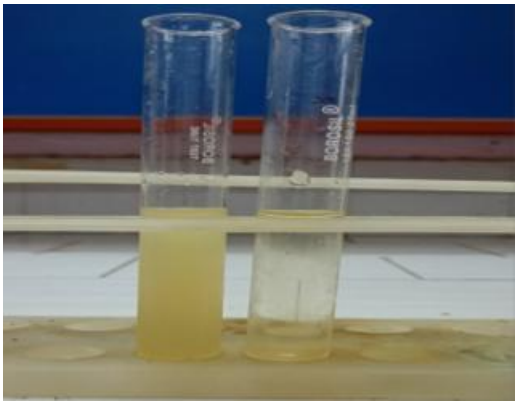

Figure 8: Clarification of fruit juice by isolate GSH-2

4. Discussion

Pectinase is utilized in various industrial processes to enhance both the quality and quantity of end products. It is essential to investigate the production process and the physiochemical characteristics of new enzymes. To further decrease overall costs, research should focus on the immobilization of pectinase enzymes for reuse purposes. Genetic engineering presents a significantly more efficient alternative, as the modifications are entirely controlled. In this manner, well-designed studies can offer valuable tools for manipulating microorganisms to generate high yields of effective and cost-efficient enzymes. Pectinase has garnered considerable attention in industrial applications, including the textile sector, fruit processing industries, oil extraction, and the fermentation of coffee and tea. (Setegn Haile and Abate Ayele 2022).

In the present study, a total of 10 different soil samples are collected from different regions of Kalaburagi. Screening for the pectin hydrolyzing bacteria is done in which formation of hollo clear zone around the colonies when flooded with iodine solution (0.1 N) observed on the pectin agar media similar results are obtained by the similar to the procedure followed by Yahya S. Alqahtani et al., (2022).

Further selection of the potent strain is done by measuring the zone of clearance and hydrolyzation where among 10 different isolates, isolate KS-2B showed highest zone of clearance and hydrolyzation that is 32mm which is somewhat similar to the result obtained by researcher Kathawut Sopalum, and Siriluck Iamtham., (2020). The isolate GSH-2, was further employed for various optimization process by using different pH and Temperature values, in which at temperature 40ºC and pH6.5were showed highest activity of production of pectinase.

5. Conclusion

From the above investigation it was found that the isolate GSH-2 is showing good pectinase activity; from this, it is concluded that the strain GSH-2 is found to be a good producer of pectinase and suitable for industrial production of pectinase when compared to other strains, as it showed potent pectinase activity in submerged fermentation and found to be useful enzyme in beverage industry for the clarification of the juice. Further, in future it can be used for industries.

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Author Profile

Sheefa Tarannum received the Bachelors of science degree from Gulbarga University, India, in 2019 and the Master's in Microbiology from Gulbarga University, India, in 2021. She passed the Karnataka State Eligibility Test in 2021 and working as Guest lecturer in Department of Microbiology, Gulbarga University from 2021 to till date.