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Screening And Molecular Identification Of Pectinolytic Microbes

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Abstract: The pectic substance is a complex colloidal acid polysaccharide with long galacturonic acid pillars, from a novel pectin-degrading bacterium that could otherwise be an agricultural waste product and that can be used for a variety of purposes. chains and glycosidic bonds. There are 7 polysaccharides and 17 monosaccharides such as d-glucuronic acid, 1-fucose, d-glucose, d-mannose and d-xylose in these pectin compound chains. Pectic acid, pectic acid, pectin, and protopectin are four pectin substances used as substrates for pectinase treatment. Although the solubility of pectic substances in water was one of the most relevant criteria used to determine that only 25% of microbial pectinase enzymes are used in the food and industrial sectors worldwide. , the market continued to grow. Pectinases are used in fruit extraction, fruit juice clarification, plant fiber refining, natural fiber degumming, and wastewater treatment. It also speeds up tea fermentation and eliminates foaming by destroying instant tea powder. Pectin in matcha. Not only is it used to brew coffee, but it is also used to remove the slime layer from coffee bars.A widely used eco-friendly tool to treat contamination, pectinase plays an important role in the treatment of industrial products plays. 16S rRNA sequence analysis was performed for molecular characterization. The 16S rRNA sequences were aligned and the constructed family tree confirmed that this strain belongs to Enterobacter hormaechei. This bacterium may play an important role in the degradation and bioremediation of vegetable and fruit wastes resulting from agricultural, industrial, and domestic activities.

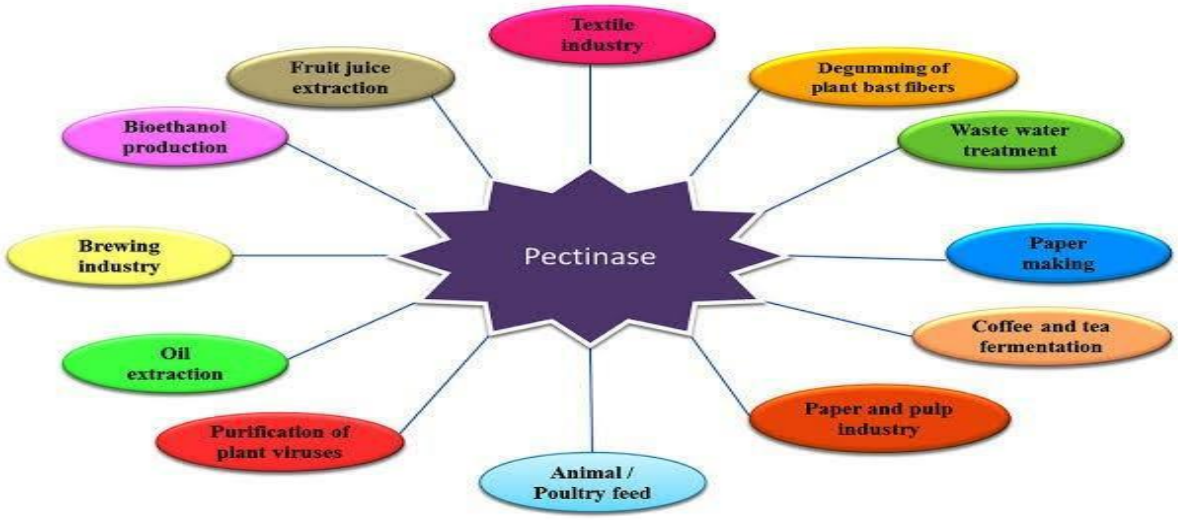
Keywords: Pectinolytic, rotten fruits and vegetables, pectinase.

Introduction

Microorganisms such as bacteria, fungi, and yeast can produce pectin-degrading enzymes. Pectinases are a group of enzymes produced by microorganisms that catalyze the breakdown of glycosidic bonds. Pectin substances are widely distributed in vegetables and fruits. Pectin is a structural acidic heteropolysaccharide found in the primary and middle lamellae and cell walls of terrestrial plants. Its main component is galacturonic acid, a sugar acid derived from galactose. Apples and citrus fruits contain pectin, which contains rhamnose. Pectinases are a group of enzymes that degrade pectin, a polysaccharide found in plant cell walls, by hydrolysis, trans-elimination, and de-esterification reactions (Ajobiewe, 2019 and Chen etal, 2019). Pectinase is used to break down and clarify wine. Pectinase can be extracted from fungi such as *Aspergillus niger*. Pectinase is used for extracting, cooking, filtering, and depectinizing fruits and vegetables, and removing the lining of lotus seeds, garlic, almonds, and peanuts (Haile, and Kang, 2019).

Enterobacter hormoechei, which produce pectinase enzyme, based on morphological, physiological and biochemical characteristics. 16S rRNA sequence analysis was performed for molecular characterization (Haritha JF, 2019). A common practice in treating rotten fruits and vegetables is to treat the pulp with an appropriate pectinase enzyme preparation. (Oliyad, A 2018). Less viscous, clearer and more nutritious juices are more desirable to consumers. In citrus juice processing, pectin enzymes help to remove cloudiness and stabilize the juice (Pasha et al., 2019). Addition of polygalacturonase had no effect on the particles, reaching the natural pH of the juice within minutes, and the amount of added enzyme affected the aggregation kinetics. Pectinase applicability in various fields of biotechnology and industry has increased the demand for commercial pectinases with novel properties and high stability. Pectinases make up approximately 10% of all enzymes used in various industrial processes. Today we devoted ourselves to the search for new bacterial cultures from various fruit, vegetable and soil samples for pectinase production on a laboratory scale. Bananas and papaya are soft and contain high levels of soluble pectin, so maceration of these fruits results in a semi-gel-like mass that is very difficult to press. (Daniella et al, 2019) The use of pectinase enzymes resulted in pulps with better pressing properties and higher juice yields.

There are some examples of pectin based emulsified products such as low fat and low cholesterol mayonnaise, low fat cottage cheese, low fat drinking yogurt, and flavoured oil containing acidified milk drinks (Kara Rogers, 2019). These are the products are prepared by substitution of full fat milk with skimmed milk, emulsified oil, and when proteins. In recent years, pectin has been used to make low- fat meat batter in in combination of inulin (Nowshad, 2018). The use of pectin in food goods as gelling agents is a lengthy custom. Pectin has been reported to form various conditions. This property of pectin is commercially exploited in the preparation of jams, jellies, and marmalades. Based on the gelling process of pectin and stabilization follows diverse mechanisms for different kinds of pectin. Chemically, pectic substances are the complex colloidal acid polysaccharides, with a backbone of galactouronoic acid residues linked by (1+4) linkages. The side chains of pectin molecules consist of L-rhamnose, arabinose, galactose and xylose. The carboxyl groups of galactouronoic acid are partially esterified by methyl groups and partially or completely neutralized by Sodium, Potassium or Ammonium ions. Pectinolytic enzymes added to macerated fruits before the addition of wine yeast in the process of producing red wine resulted in the improve visual characteristics as compared to the untreated wines.



Materials and Methods:

Sample collection: For the collection of pectinases producing bacterial strains partially decayed fruits such as rotten orange peel, papaya peel, rotten guava, potato, lemon and one soil sample were taken in sterile polythene bags from the local markets and till further proceedings, sample were stored at 4℃.

Direct Plaing Methd: Rotten fruits and vegetable samples were serially diluted and spread plate on pectinase screening agar media (PSAM) 0.3g/100ml, (NH4)2HPO4, 0.2g KH2PO4, 0.3g K2HPO4, 0.01g Mgso4, 2.5g agar, 1g pectin and Ph 5.8 and keep it for auto clave. The orange peel and all other samples which has mentioned in 4.1 The rotten oranges were used to isolate the pectinase producing bacterial strains. 1g of rotten orange pulp was added into sterile distilled water and mix it well. A total of 200µl of dilution was spread on the plates of pectinase agar medium (PAM) with 1% pectin as a sole source of carbon bacterial growth. These plates were incubated for 24 to 48hrs at 35℃. The bacterial colonies appeared on plates were further purified medium under the same condition.

Identification of Effective Microbe : At the end of the incubation period, streak colonies using pectinase agar to find clear zones. 2.5% pectin, 10 g/l selective peptone, 5 g yeast extract, 5 g NaCl and 15 g agar, PSAM were poured into Petri dishes and colonies were streaked with pectinase culture.

Pure culture Slants : Different colonies were randomly picked from the countable plates (PSAM for isolation of bacteria, nutrient agar for pure cultures) and purified by repeated streaking on the respective media. Pure culture of each group of bacteria and then streaked on slants of respective media. Composition of pure culture media was g/L, 5g peptone, 3g yeast extract, 5g NaCl, 16g agar and distilled water and pH 6.8 - 7. Ten isolates were collected from different locations, 6 isolates were identified as effective microbes, colonies were streaked on nutrient agar media and stored at 4℃ for further study.

Effect of Incubation Temperature : Each pectinase producing bacterial strains were carefully spot inoculated on PSM agar plate and were incubated at different temperature i.e., 37℃, 40℃, 45℃, 50℃, 55℃, 60℃, 65℃, 70℃ and 75℃ for 24hrs. Enzyme index was determined for each strain.

Effect of Substrate Concentration : Strains were spot inoculated on PSM plates with varying concentration of pectin (g/L): 5, 10, 15, 20 and 25. All plates are incubated at 37℃ for 24hrs. Pectinase screening plate assay by calculation of enzyme index for each strain was performed (Sagar A 2019).

Effect of Incubation Tie : Cultures were spot inoculated on PSM agar plates. Inoculated plates were incubated at 37℃ for different incubation time i.e., 24hrs, 48hrs, 72hrs, 96hrs and 120hrs. Pectinase screening assay was performed to determined enzyme index.

Pure cultures Biochemical Characterization : The biochemical tests were performed for the characterization of pectinase producing strains were gram staining, colonial and morphological characteristics.

Primers : Specific primers were needed by amplifying a fragment of pel A as showed. Primers were provided in a lyophilized form and dissolved in sterile nuclease-free water to give a final concentration of 100pmol/µl. Afterward, they were stored in a deep freezer until use.

Primer used in pel A gene:
Forward primer – 5’ CCTTCAGCCATCCGTTCTTCT 3’
Reverse primer – 5’ TCGCGTACGAAGTCGACCTT 3’
Product size - 118 base pairs

PCR amplification: PCR technique was performed by using genomic DNA of Enterobacter hormoechei isolates as a template with the specific primers shown on above. Each primer consists of 10µl of master mix, 1µl of forward primer, 1µl of reverse primer, 6µl of Rnase free water, 2µl of template DNA, and 10µl of final volume. PCR program for pel A primer included initial denaturation at 94℃ for 5 min followed by 35 cycles of denaturation of 94℃ for 1min, at 52℃ for 40 sec and extension at 72℃ for 50sec. PCR products were characterized by gel electrophoresis on 1% agarose gel. After electrophoresis, the gel was visualised in gel documentation system and picture was taken.

Results and Discussion :

Isolation of Individual Colonies: First 10 different bacterial stains were isolated from different fruits and vegetable rotten peels through primary screening method (Fig.1 and Table. 1) (Govindaraji et al, 2019). These isolates were further screened for pectinase production capability by employing the secondary screening method. Six bacterial isolates which as higher pectinase activities were identified to be *Enterobacter hormoechei*. The analysis of pectinase synthesis and application in fruit juice clarification was performed by using one of the bacterial strain, *Enterobacter hormoechei*. As the result, the maximum crude pectinase enzyme activity from *Enterobacter hormoechei* was found in 72 hours of incubation time, at the optimum temperature of 35℃, pH 8, using 1% of pectin substrates as carbon source. Finally, the percentage yield and the clarity of apple, lemon, and orange fruit juices were investigated using both crude and partially filtered pectinase.

Fig. 1 Biochemical tests

Table -1 Result interpretation of different bio chemical test

SI NO	Name Biochemical test	Reagent name	Colour appearance	Result
01	Indole test	Kovac's reagent	Pale pink	Positive
02	Methyl red test	Methyl reagent	Red colour	Positive
03	VP test	VP-A,VP-B	Absence of pink red colour	Negative
04	Citrate test	_	Green to blue	Positive
05	Catalase test	H2O2 solution	Immediate bubble formation	Positive
06	TSI test	_	Red slant, yellow butt, Black and gas production	Positive
07	Nitrate reduction test	Sulphonic acid reagent, α-Naphthylamine reagent, Zinc dust powder	Red colour	Positive
08	Gelatine hydrolysis test	_	No solidification	Positive
09	Lactose fermentation test	_	No bubble formation	Negative

Conclusion:

The fruits and vegetables contain a high amount of pectin, the extraction of fruit juice has historically resulted in a cloudy unappealing colour and high viscosity (Bharadwaj and Udupa, 2019 and Garg and Mahajan, 2016). Researchers studied fruits and vegetable wastes that were found to contain four distinct bacterial strains (Singh et al., 2019). One strain was classified as a *Serratia marcescens* based on the morphological and biochemical characteristics (Priyanka et al, 2019). It's important to do more research on this enzyme to make sure and improve the efficiency of the bacteria for using the fruit industry. The pectinase is isolated from the bacterium "*Serratia marcescens*" could clear fruit juices (Bijesh and Denoj 2017). This pectinase needs to be studied more to make sure it works better in the fruit industry and other businesses.

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