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Isolation, Production and Characterization of Pectinase Enzyme from Fungal Sources: A Review

[1]Sweata Dey, [2]Ayantika Laha, [*3]Manisha Maity

[1]M.Sc. Student, Department of Food and Nutrition, Swami Vivekananda University, Barrackpore, W.B., India.
[2]M.Sc. Student, Department of Food and Nutrition, Swami Vivekananda University, Barrackpore, W.B., India.
[*3]Assistant Professor and Head, Department of Food and Nutrition, Swami Vivekananda University, Barrackpore, W.B., India.

*Corresponding author's E-mail: manisham@svu.ac.in

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Abstract

Pectin, a plant cell wall polysaccharide, is broken down by the de-esterification, hydrolysis and trans-elimination procedure using the pectinase enzyme. They are also known as pectic enzymes and include the commercial pectinases pectolyase, polygalacturonase, and pectozyme, which have all undergone extensive research. This study aimed to know the procedure of isolation, screening and characterization of pectinase-producing fungi from different food sources and their application in juice extraction, wine clarification, coffee extraction, tea, vegetable oil extraction, cocoa, etc. Pectinase enzymes are obtained naturally by a variety of insects, fungi, yeasts, plants, bacteria, and microorganisms; moreover, animal or human cells cannot make pectinase enzymes. The present review discusses the production, classification, application and microbial sources of the pectinase enzyme.

Keywords: *Pectin, Pectinase, Microbial Pectinase, Application in food industries*

1. Introduction

According to their mode of action, pectinases are a group of enzymes that break down pectic material. Methoxy groups, for instance, are removed by methylesterases from strongly or partially esterified galacturonan. Polygalacturonases catalyse the release of galacturonic or digalacturunic acid residues by hydrolyzing glycosidic linkages in a random manner (endopolygalacturonase) or from the nonreducing end of homogalacturonan (exopoly- glacturonases) (KC et al., 2020). Pectinolytic enzymes, commonly known as pectinases, can also be divided into groups based on the substrate they work on and how they function.

Lyases, which are further divided into pectatelyases or pectin lyases, polygalacturonases, which are further divided into endo- and exo-polygalacturonases, and pectin methylesterases (Oumer et al., 2018; Garg et al., 2016).

Pectic compounds are the collective name for the substance that pectinase hydrolyzes. One of the primary components of cereals, vegetables, fruits, and fibres, pectin is also described as a polysaccharide that is rich in extremely important sugars, galacturonic acid, and methanol as its key components. It also has a high molecular weight heterogeneous and acidic structural polysaccharide (Haile and Ayele, 2022).

To produce pectinase, yeast species including*Saccharomyces fragilis*, *Saccharomyces thermantitonum*, *Torulopsis kefyr*, *Candida pseudotropicalis var lactosa*, and *Candida pseudotropicalis c*an break down pectin-containing materials (Kavuthodi and Sebastian, 2018). By generating pectinolytic enzymes, certain fungal species may break down pectic materials. *Aspergillus Niger, Aspergillus awamori, Penicillium restrictum, Trichoderma viride, Mucor piriformis,* and *Yarrowia lipolytica* are the most common and effective fungi in the production of pectinase. These organisms play a significant role in both submerged and solid-state fermentation for the production of various industrially significant products (Nawaz et al., 2018) The synthesis of pectinase by *Erwinia species, Pseudomonas fluorescens, Bacillus, pseudomonas,* and *Micrococcus* has a good potential to breakdown pectin (Geetha et al., 2014; Sohail and Latif, 2016; Kavuthodi and Sebastian, 2018).

The present aim of this project is to the application of new enzymes with desirable biochemical and physicochemical characteristics and low-cost production in commercial processes has always been regarded as essential research. Keeping all the advantages into consideration, the work will be done to isolate and screen pectinase-producing fungi from the soil samples, to optimize different parameters for maximum enzyme production and evaluate the enzyme activity with various parameters, and to evaluate its potentiality in juice clarification.

2. Pectin enzyme and its classification

Pectin-containing substrates are broken down by a class of enzymes known as pectinolytic enzymes (Blanco et al., 1999). Pectic enzymes are abundant in nature and are made by a variety of organisms, including bacteria, yeast, fungus, and plants (Gummadi et al., 2002; Whitaker, 1990). Pectic enzymes are essential to plants because they aid in cellular expansion, elongation, and fruit ripening (Shet et al., 2018). Pectinase enzymes are categorized as belonging to the hydrolase group by the international union of biochemistry and the enzyme commission (Gurung et al., 2013).

2.1 Protopectinase

Protopectin is the insoluble component of pectic compounds, and it requires additional solvents to be broken down. One type of pectinase, protopectinase, breaks down the insoluble protopectin found in unripe fruits to produce highly polymerized soluble pectin. Pectinosinase is another name for this enzyme (Tapre and Jain, 2014).

2.2 Pectin Methylesterases

The removal of this methoxyl group from pectic substances is carried out by pectin methylesterases, which results in the production of pectic acid and methanol. As a carboxylic acid esterase that is a member of the hydrolase family of enzymes, pectin methylesterase is also referred to as pectin pectylhydrolase, pectinesterase, pectin demethoxylase, pectase, and pectolipase (Parmar and Rupasinghe, 2013).

2.3 Polygalacturonase

Because it participates in the depolymerization process, polygalacturonase, one of the pectinase classifications, is also known as depolymerase. The pectinolytic enzymes known as polygalacturonases catalyse the hydrolytic cleavage of the polygalacturonic acid chain when there is water present (Rebello et al., 2017; Kant et al., 2013). According to Patidar et al. (2016) investigation, polygalacturonases can be divided into two groups. These are exo- and endo- polygalacturonase, respectively. Oligogalacturonic acids are produced when endopolygalacturonase hydrolyzes polygalacturonic acids. Pectic acids are hydrolyzed by exo-polygalacturonase, releasing mono-galacturonate.

3. Sources

Citrus peel and apple pomace have traditionally been used as sources of pectin for commercial purposes. Due to its high pectin concentration and appealing colour features, orange peel has frequently been the favoured material for pectin production. Sunflower and sugar beetroot are the other sources of pectin, though. Apple pomace, dried citrus peel, and leftovers from the processing of fruit juices are the main basic materials used to make pectin ((Alkorta et al.,1994; Blanco et al., 1999).

3.1 Microorganisms for pectinase production

In nature, pectinase enzymes are found in abundance. Plants, bacteria, fungus, yeasts, insects, nematodes, and protozoa are the principal hosts for them (Lang and Domenburg, 2000). Numerous significant bacteria, fungi, and yeasts are adept at breaking down pectins to create pectinases (Wong et al., 2017).

3.2 Pectinolytic Fungi

The most well-known and effective fungi for producing pectinase are *Aspergillus niger, Aspergillus awamori, Penicillium restrictum, Trichoderma viride, Mucor piriformis, and Yarrowia lipolytica*. These fungi play a significant role in both submerged and solid-state fermentation for the production of numerous industrially significant products (Nawaz et al., 2018). *Penicillium janthinellum*, a strain of pectinase-producing bacteria, was isolated by Kumari et al., 2013 from soil and has since been identified as producing considerable levels of exo- polygalacturonase, an extracellular pectinase. One of them, Tetracoccosporium species, was discovered to generate pectinase well and exhibit pectinolytic activity in a 20-mm clearance zone around the colonies (Aminzadeh et al., 2007). Different *Aspergillus Niger* strains' pectinase production was examined by Khairnar et al. (2009). They discovered that *Aspergillus Niger* had a 4.5 mm zone of clearance for pectin hydrolysis (Khairnar et al., 2009).

3.3 Pectinolytic Yeasts

Saccharomyces fragilis, Saccharomyces thermantitonum, Torulopsis kefyr, Candida pseudotropicalis var, lactosa, and *Candida pseudotropicalis* are yeast species that can break down pectin materials in the pectinase synthesis processes, according to Kavuthodi and Sebastian (Kavuthodi and Sebastian, 2018). Additional yeast species for the synthesis of pectinase are also included in the other report. These species include Saccharomyces species, *Cryptococcus species, Aureobasidium pullulans, Rhodotorula dairenensis, Kluyveromyces marxianus, Geotrichum klebahnii*, and *Wickerhanomyces anomalus* (Hassan and Ali, 2016) techniques, it was discovered how well *Bacillus licheniformis* produces pectinase. Fermentation was used for the secondary screening after the potassium-iodide flooding procedure for the initial screening (Rehman et al., 2015).

3.4 Pectinolytic Bacteria

Pectinase is produced by bacteria such *as Erwinia species, Pseudomonas fluorescens, Bacillus, Pseudomonas,* and *Micrococcus* (Geetha et al., 2014; Sohail and Latif, 2016; Kavuthodi and Sebastian, 2018). According to reports, pectinolytic *Bacillus licheniformis* was isolated from the decaying vegetable. Using primary and secondary screening.

4. Production of Selected Pectinase from Microorganisms

Generally speaking, naturally occurring pectinases are found in either plants or microorganisms; however, their isolation and commercial synthesis from microbial sources will meet the industrial needs through a large-scale fermentation-based production process. The crucial steps in optimising each enzyme are the initial isolation and identification of selective microorganisms, screening of selective media, and production process using flask culture or scale-up culture [fermentation process] (Oumer and Abate., 2018; Bharadwaj and Udupa, 2019).

The commercially available pectinase enzymes are made either through submerged fermentation (SmF) or solid-state fermentation (SSF), depending on the specific fungus or bacterium used (Sandri and Silveira, 2018). For the pectinase manufacturing process to be optimized, particular microorganisms and a suitable medium must be chosen or adapted, along with some external environmental conditions (Satapathy et al., 2020).

5. Application in food industry

A set of enzymes known as pectinases catalyse several events in the pectic materials found in plant cell walls. These include the transeliminative cleavage of 1,4-D-galacturonan methyl ester by pectolyase, the hydrolysis of 1,4-glycosidic linkages by polygalacturonase, and the deacetylation and demethoxylation of pectin-by-pectin esterase (Sudeep et al., 2020).

The use of pectinolytic enzyme varies depending on the physical circumstances that are present. Pectinases have been utilised in a variety of traditional industrial processes, including the extraction of tea, coffee, and oils, the processing of plant fibres for textiles, and the treatment of industrial effluent.

In the fruit juice industry, pectinases are used to break down pectin (depectinization) in the cell walls of fruits, improving juice extraction, flavour, clarity, filterability, and total yield (Sudeep et al., 2020; Tapre et al., 2014; Anand et al., 2017).

Pectinases can be utilised in the beverage sector during the curing stage of wet processing for coffee production as this increases yield, improves aroma and flavour, and shortens processing time. Pectinases can also be utilised in cocoa processing facilities to ensure that the mucilaginous layers of cocoa beans are completely removed, enhancing the quality of the resulting coffee (Oumer and Jeilu, 2017).

In order to ensure a somewhat quicker fermentation process when making tea, tea leaves are treated with pectinases to lower their pectin concentration (Suhaimi et al., 2021).

5.1 Extraction of fruit juice

Fruit juice clarity and extraction represent pectinases' most significant industrial application. The pectins contribute to the viscosity and turbidity of fruit juice. An amylase and pectinase combo clarifies fruit juices (Blanco et al., 1999).

Pectinase is frequently employed in the production of fruit and vegetable juices. In order to protect the integrity of plant cells, these companies commercially manufacture a variety of juices, such as sparkling clear juices, cloudy juices, and unicellular goods (Rehman et al., 2021). By adding these enzymes to fruit pulp, pectin is broken down, reducing viscosity and making fruit juice easier to handle. These enzymes are crucial for the maceration, solubilization, and clarity of fruit pulps. Traditional methods for clarifying

pectin-containing juice include centrifugation to remove suspended solids, enzymatic treatment for depectinization, and fining agents such bentonite and gelatin to remove haze and actinomycetes (Bhardwaj et al., 2017).

Fruit juice is naturally cloudy because pectin polysaccharide (Sharma et al., 2017) (pectin, lignin, hemicellulose, cellulose, and starch), tannin, protein, and metals (Vaillant et al., 2001) is present in it (Sharma et al., 2017). Pectinases have been combined with other enzymes, including as cellulases, arabinases, and xylanases, to improve the effectiveness of pressing fruits for juice extraction. Pectinase reduces the viscosity of fruit juice during the clarifying process by breaking down the pectin material in the juice and enhancing the pulp's pressing capacity, while also dissolving the jelly structure and boosting fruit juice yields (Al-Maktari et al., 2019).

The release of phenolic compounds from the fruit skin is further aided by the use of pectinases in the preparation of fruit juice (Sharma et al., 2013). This process raises the phenolics content by up to 15%, demonstrating the fruit beverages' strong antioxidant properties (Aliaa et al., 2010). These phenolic substances significantly reduce oxidative stress and show preventive effects against cancer and cardiovascular disease (Miller and Rice-Evans, 1997).

5.2 Clarification of wine

The main functions of pectinolytic enzymes in the wine-making process include assisting with extraction, increasing juice output, facilitating filtration, and enhancing flavour and colour. Pectinases are used in the wine-making process to speed up the maceration process, increase juice extraction yield, quicken the filtration process, and enhance flavour and colour. Pectinases were used to macerate the fruits before inoculating the alcoholic fermentation. This method raises the standard of the wine (Samanta, 2021).

As processing aids for pectin degradation, which settled down organic particles in suspension, commercial pectinolytic enzymes are utilised. Juice output and clarity increased with the introduction of pectinolytic enzymes, and the nutrients, original colour, and flavour were also preserved. When making clarified juices, a combination of pectinase, cellulase, and hemicellulase enzymes was successful in reducing viscosity and improving filterability (Tapre et al., 2014).

Fruits have been pectinase-macerated prior to the inoculation of alcoholic fermentation. This procedure improves the wine's quality (Praveen and Suneetha, 2014). Pectic enzymes are added to the fruit-crushing step of the winemaking process to increase the amount of free-flow juice and shorten the pressing time. Additionally, it facilitates juice filtration and clarity as well as enhances the chromaticity and stability of red wines (Lavanya and Gayatri, 2021).

5.3 Preparation of jam and jellies

A surplus of sugar is added for gelation when making jams and jellies. Pectin esterase reduces the need for sugar because it changes highly methoxylated pectins into less methoxylated ones, which increases the gelation property in conjunction with calcium. This enzyme is used to make sauces, soups, compotes, jams, and jellies (Kubra et al., 2018).

5.4 Tea, cocoa, coffee concentration and fermentation

In general, a large range of non-volatile substances are present in tea leaves, including polyphenols, flavonols and flavonol glycosides, flavones, phenolic acids and depsides, amino acids, chlorophyll and other pigments, carbohydrates, organic acids, caffeine and other alkaloids, minerals, vitamins, and enzymes (Chaturvedula and Prakash, 2011).

Pectinases speed up tea processing and diminish the foaming capability of instant tea powders by degrading the pectic compounds, which are found in tea leaves in amounts ranging from 5-6 % in the middle lamella of their cell walls (Marimuthu et al., 2000).

The fermentation process for coffee also makes use of pectinase. These hard covers of coffee beans are known as mucilage and encircled the internal structures of the coffee bean. Additionally, the mucilage contains sticky and gelatinous qualities that make it difficult to brew drinkable coffee. Before using the coffee bean, the mucilage coat is removed from it using alkaline pectinase during the procedure (Praveen and Suneetha, 2014).

5.5. Vegetable oil extraction

Oil extraction from a variety of sources, including flaxseed, olives, dates, and others, has been extensively studied using pectinase and other cell wall degrading enzymes (CWDE) (Anand et al., 2020). Pectinases

are enzymes that interfere with pectin's emulsifying properties, which prevent oils from being extracted from citrus peel extracts, allowing for the extraction of citrus oils, such as lemon oil (Oumer, 2017).

The greatest yield of oil uptake is increased when cellulases, hemicellulases, and pectinases are used in combination during the oil extraction process. Additionally, this procedure increases the oil's polyphenolic and vitamin E content and enriches the amount of antioxidants (Kashyap et al., 2001; Hoondal et al., 2002; Iconomou et al., 2010).

6. Conclusion

The application of new enzymes with desirable biochemical and physicochemical characteristics and low- cost production in commercial processes has always been regarded as essential research. As the scope of commercialization of enzymes is growing in the world market, microorganisms have become highly sought-after resources for enzyme production. In today's world pectinolytic enzymes have gained a huge market value and have been used in numerous food industry. Using microbial enzyme encourage the use of green technology. This review has been focuses on fungal pectinase enzymes that have been shown to be used in various industries.

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