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Diversity and Industrial Applications of Fungal Pectinases

Asheesh Kumar Mishra[1], Kaushal Kishor Mishra[1], Chaudhary Shalu Singh[2], Manyata Sharma[1], Deepak Chand Sharma[1*]

[1*]*Department of Microbiology, DSMNR University, Lucknow - 226 017, Uttar Pradesh, India.*

[2]*Research Scientific Officer, Mohan Meakin Limited, Mohan Nagar, Ghaziabad - 201 007, Uttar Pradesh, India.*

[*]*Department of Microbiology, DSMNR University, Lucknow - 226 017, Uttar Pradesh, India.*

**Corresponding Author Email: dcsharma@dsmnru.ac.in*

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ABSTRACT

Pectins play a vital role in maintaining the structure and firmness of plant tissues, providing resilience to cell walls and protecting plants from drought and wilting. Fungi possess a diverse group of extracellular pectinolytic enzymes, known as pectinases, which serve as valuable tools for infecting their host plants or drawing energy by degrading plant materials. Pectinases specifically target and break down pectin and pectic substances into monomers with diverse modes of action. In food processing, winemaking, paper, tea, coffee and textile industries, pectinases are widely recognized as the commonly used enzymes. They are employed in developing new products, enhancing production of the existing products, physical, chemical and sensory properties and increasing overall yield. This review aims at targeting the biochemical characteristics of fungal pectinases, with specific focus on their relevance in different industries. Additionally, it provides a comprehensive overview of the applications of fungal pectinases in various industrial processes.

Keywords: Pectinases, Polygalacturonase, Fungal pectinases, Alkaline Pectinases, Acidic Pectinases, Fiber retting,

INTRODUCTION

Enzymes are essential proteins that catalyse countless chemical reactions the living beings by reducing the energy required for them to occur. Exploitation of enzymes in industrial production gave rise to biotechnological processes having several advantages such as reduced production costs, lower energy consumption and less pollution (Haile *et al.*, 2022; El-Enshasy *et al.*, 2018).

Approximately 25,000 natural enzymes are estimated to be present in living beings; 25% of these are known and more than 120 of them are being used in various industrial applications. Enzymes play an increasingly important role in industrial production processes (Garg, 2016; Yang *et al.*, 2020). The global enzyme market valued at 6.95 billion US$ in 2022 and is expected to grow at a compound annual growth rate (CAGR) of 6.4% from 2023 to 2030 (Grand View Research 2023). The global food enzyme market reached a value of almost 2.2 billion US$ in 2020. The industry is expected to grow at a CAGR of 6.4% between 2021 and 2026 to reach a value of almost 3.1 billion US$ by 2026 (Food Enzymes Market - Trends; Growth and Forecast Analysis 2021; Kotnala, 2021). The major share of industrial enzymes lies in detergent, food, feed, paper and textile industries **(Figure 1).**

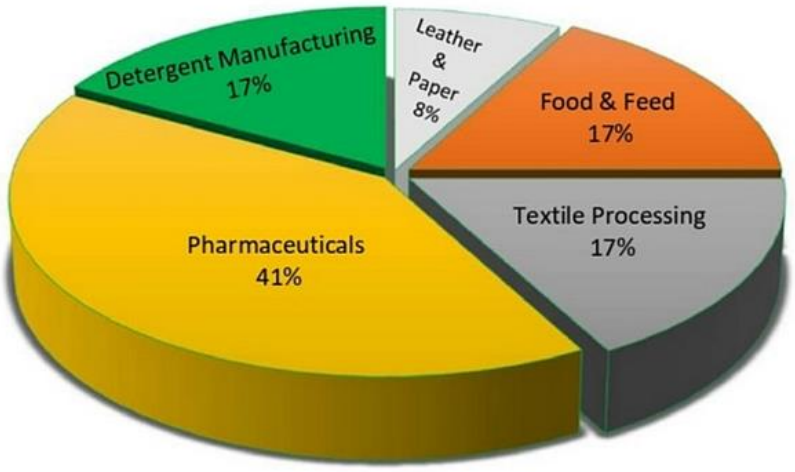

Figure 1: Market share of enzymes in various industries.

The Indian food enzymes market is projected to register a CAGR of 3.8% during 2020-2025. The Indian market reached Rs. 2,525 crores in the last financial year. India comprises a large market for biotech-based products and most of these products are produced by DSM, Novozymes and Advanced Enzyme Technologies (Robledo *et al.,* 2020; Sharma *et al.,* 2023).

Enzymes can be found in various forms in living organisms, including animals, plants and microbes. The industrial sector requires large quantities of enzymes, which cannot be met by the limited supply from animal and plant sources (Venkatanagaraju *et al*., 2019). Therefore, microbial sources have gained greater attention in the enzyme production (Sharma and Satyanarayana, 2006; Sharma *et al.,* 2023). The microbes are known to produce various enzymes like pectinases, proteases, phytases, cellulases and several others.

Microbes produce a number of pectin degrading enzymes, which play an essential role in the degradation of pectic substances and have extensive applications in food processing, biological degradation of plant materials, fermentation and food spoilage (Kohli and Gupta, 2015).

Pectins, the major constituents of cereals, vegetables, fruits and fibers, are complex high molecular weight heterogeneous and acidic structural polysaccharides. They are present in the middle lamella of plant cell wall as a thin layer (Begam *et al.,* 2020; Nawaz *et al.,* 2018). It acts as an adhesive extracellular material attached to cellulose microfibrils, surrounded by a matrix of hemicelluloses and proteins, **(Figure 2 a, b)** acting as 'cementing' agent (Sharma *et al.,* 2023).

Pectin is synthesized in Golgi apparatus as uridine diphosphate-D-galacturonic acid during early stages of plant growth (Wong *et al.,* 2019). The pectic polysaccharides received attention due to their significant role in ripening of fruits and widespread application as a gelling agent in food processing industries (Verma *et al.,* 2017). The presence of pectic material also influences the texture of fruits and vegetables, one of the most characteristic changes that occur during the ripening of fleshy fruits is softening that influences the texture and taste as well. In unripe fruits, pectin is bound to cellulose microfibrils in the cell walls and called protopectin. The protopectin is an insoluble polysaccharide and therefore, confers rigidity to the cell (Mao *et al.,* 2021).

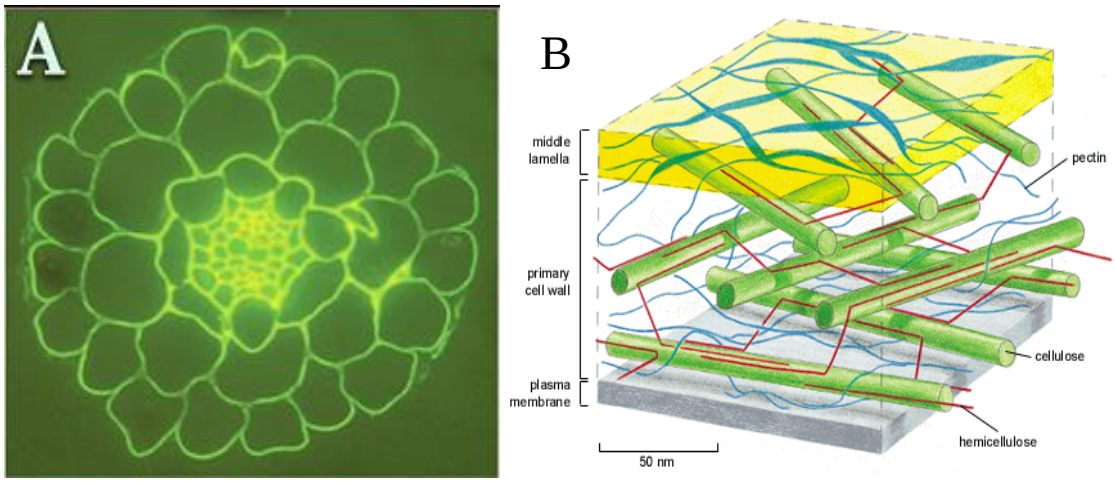

Figure 2: Distribution and structure of pectin in plants. **A,** Immunofluorescence micrographs showing the distribution of pectin epitopes in roots of *Arabidopsis* using monoclonal antibodies (Transverse section) (Bacic 2006); **B,** Adhesive extracellular material (pectin) attached to cellulose microfibrils, surrounded by a matrix of hemicelluloses and proteins (Robledo and Vázquez 2020).

Pectin molecules consist of several hundred to thousands of D- galacturonic acid units that are linked together by α-(1,4) linkages in axial arrangement, which forms a trans-1,4-polysaccharide and it has a tendency of coiling around the screw axis (Liu *et al.,* 2018). The carboxyl group of D-galacturonic acid unit in pectin is partially esterified with methanol or neutralized by monovalent or divalent cations like K^+, Na^+ and Ca^{2+}. The degree of esterification is 100% when the methoxyl content is 16.32% (Ali *et al.,* 2023). Sometimes the hydroxyl groups at C3 or C4 positions are also acetylated, and the degree of acetylation may vary from 0.18 -2.5% (Nighojkar *et al.,* 2019). The acetyl groups are important as they determine the gelling properties of the polymer. These pectic substances are degraded and solubilized by naturally occurring enzymes called pectinases in the fruits leading to softening of the fruits during the process of ripening (Guan *et al.,* 2020; Hao *et al.,* 2023).

The enzymes responsible for breaking down pectins are widespread and have diverse modes of action. Pectinolytic enzymes include a group of enzymes that are able to catalyse the breakdown of pectin containing substrates (Abdullah *et al.*, 2021). These enzymes have various industrial applications such as clarification of fruit juices, improvement of yield and flavour of alcoholic beverages, scouring of natural fibers in textile industry and improvement of quality of paper products.

PECTIC SUBSTANCES AND THEIR STRUCTURES

Pectic substances are a group of complex polysaccharides which are made up of galacturonic acid and other sugar residues such as rhamnose, arabinose, xylose, galactose. They are categorized into three types: homogalacturonan, rhamnogalacturonan-I and rhamnogalacturonan-II (Sharma and Satyanarayana, 2004; Lu *et al.*, 2018) (**Figure 3**).

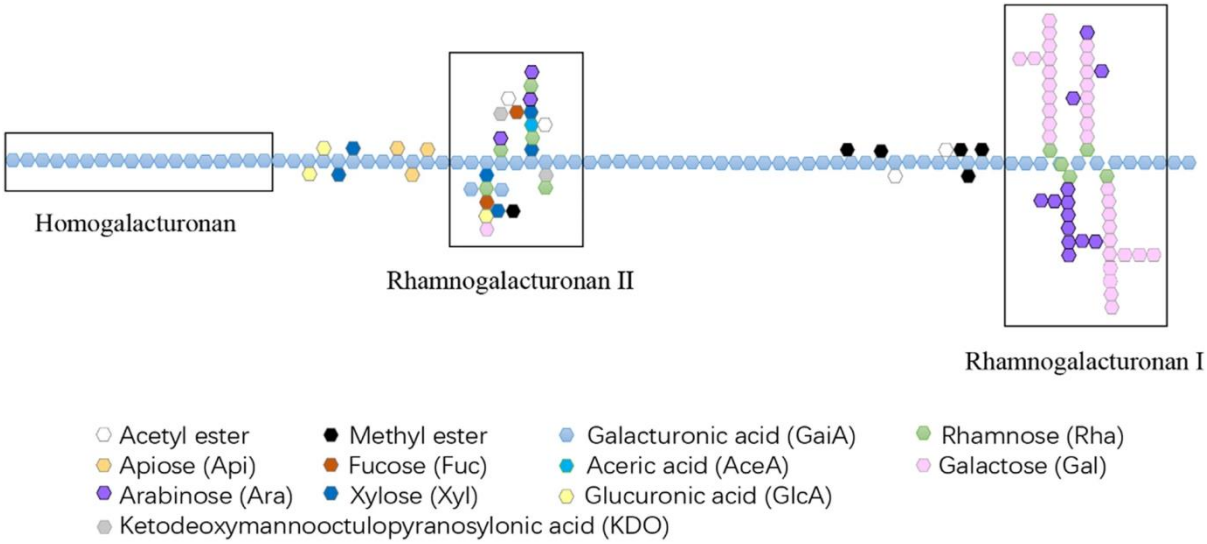

Figure 3: The structure and composition of pectin (Zheng *et al.*, 2021).

Homogalacturonan (HG) is a linear polymer of α-1,4-linked galacturonic acid. The degree of esterification (DE) of HG is an important structural feature that refers to the degree to which carboxyl groups on the galacturonic acid residues are esterified with methanol or other alcohol groups (Waldron and Faulds, 2007; Doan *et al.*, 2021). The distribution of the ester groups along the HG chain affects its physical properties and biological functions. Low-esterified HG (LM-HG) with a DE of less than 50%; this is commonly found in young tissues that is highly soluble in water. High-esterified HG (HM-HG) with a DE of more than 50% is more commonly found in mature tissues and is less soluble in water (Sénéchal *et al.*, 2014). The degree of esterification affects the ability of HG to form gels or to interact with other components in the cell wall. HG plays an important role in the physical and mechanical properties of plant cell walls such as cell adhesion, cell expansion and plant growth. It is also involved in various biological processes like plant-microbe interactions, pathogen defence and fruit ripening. HG is widely used in the food industry as a gelling agent, thickener and stabilizer, particularly in the production of jams, jellies and other fruit-based products (Wolf *et al.*, 2009; Daher and Braybrook, 2015).

Rhamnogalacturonan-I (RG-I) is a branched polysaccharide that contains alternating residues of galacturonic acid and rhamnose. The rhamnose residues can be substituted with various side chains, such as arabinan, arabinogalactan and galactan. The type and frequency of these side chains can vary depending on the plant species and tissue type (Naran *et al.*, 2008). Additionally, rhamnose constitutes a small component of pectin backbone and is present as α-L-rhamnopyranose. They are present in various segments of structure: α-D-galactopyranosyluronic acid-(1→2)-α-L-rhamanopyranosyl-(1→4) galactopyranosyluronic acid. The presence of rhamnose leads to the formation of a T-shaped 'kink' in the linear chain. Other neutral sugars like L-arabinofuranose and D-galactopyranose occur most frequently in the side chains, whereas D-glucopyranose and L-fucopyranose are less common throughout the structure (Mao *et al.*, 2019). D-Adipose, 2-O-methyl-D-xylose and 2-O-methyl-L-fucose are wide spread constituents of pectins present in minute quantities (Mohnen, 2008). These side chains are composed of neutral sugars that give the 'hairy' edifice to rhamnogalacturonan portion of the pectin (Kaczmarska *et al.*, 2022).

Rhamnogalacturonan II (RG-II) is a complex polysaccharide that contains a core of disaccharide

repeating units of α-1,4-linked galacturonic acid and α-1,2-linked rhamnose, which is extensively branched with arabinan, galactan and apiofuranosyl residues (Mao *et al.,* 2019). RG-II is considered to be one of the most complex plant cell wall polysaccharides known to date. RG-II is thought to have important biological functions in plant development, including cell expansion and division and plant-microbe interactions (Gawkowska *et al.,* 2018). The complex structure of RG-II has made it difficult to study and its functions and interactions with other components of the plant cell wall are not yet fully understood. However, recent advances in analytical techniques, such as mass spectrometry and NMR spectroscopy, have provided new insights into the structure and biological functions of RG-II. RG-II has also garnered interest in the development of new biomaterials for various applications, including drug delivery and tissue engineering (Zhang *et al.,* 2021).

Nomenclature

In 1944, the Committee for the Revision of Nomenclature of Pectic Substances, a former subdivision of American Chemical Society, defined pectic substances as those complex colloidal carbohydrate derivatives, which occur in plants and contain a large proportion of anhydro galacturonic acid units that are in a chain like combination (Wegener *et al.,* 2015). The carboxyl groups may be partially esterified by methyl groups and partially or completely neutralized by one or more bases. The committee has defined complex pectic substances as described below:

Protopectin

Protopectin is water-insoluble pectic substance observed in the cell walls of plant tissues, besides ripening of fruits. On hydrolysis it produces pectin and pectic acids **(**Garg *et al.,* 2013; Minten *et al.,* 2014). The insoluble nature of the protopectin depends on the polymer size and the presence of divalent cations like Ca^{2+}.

Pectinic acids

These are the colloidal polygalacturonic acids with different proportions of methyl ester groups. Pectinates are normal or acidic salts of pectic acids; pectic acid alone has the unique property of forming a gel with sugars and acids or if it has reasonably low methyl content with compounds such as calcium salts (Oumer *et al.,* 2017).

Pectin or pectins

The pectin refers to water-soluble polymeric material, which has various degrees of esterification with methanol and can form gels with sugar and acid under appropriate conditions (Hoondal *et al.,* 2002).

Pectic acid

Pectic acid is a category designation used to the pectic substances mostly composed primarily of colloidal polygalacturonic acids and necessarily free of methyl ester groups **(Table 1).** Pectic acids are mostly formed after tissue breakdown by the action of pectin methylesterases. Natural or acidic salts of pectic acid are called pectates (Sharma and Satyanarayana, 2006).

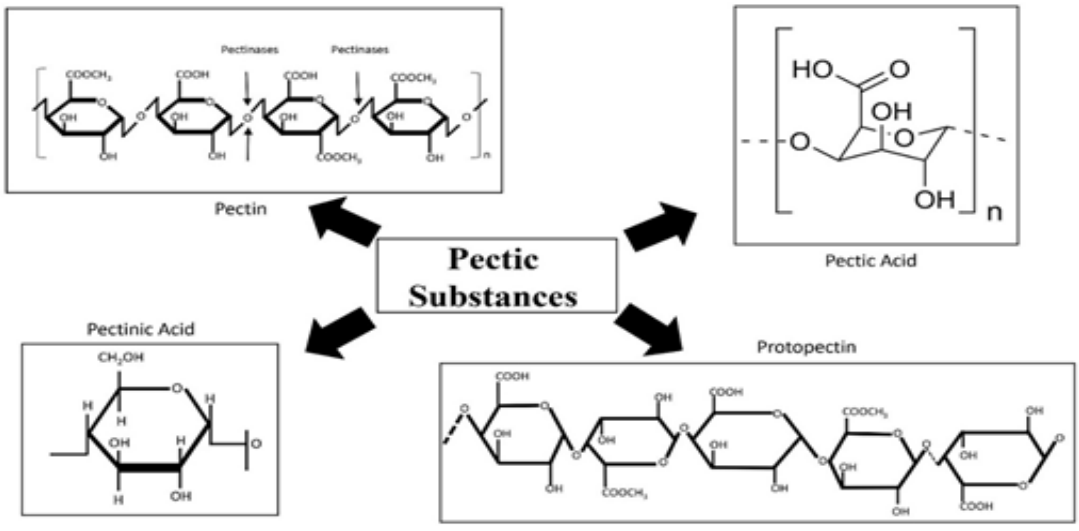

Figure 4: Structure of pectin and its functional groups.

Table 1: Types of pectic substances

S. No.	Type of Pectin	Description	Sources
1	Pectinic acid	Having variation in methoxyl content and under suitable conditions can form gel with sugar.	Garg *et al.,* 2013
2	Pectic acid	Galacturonans lack methoxyl group, soluble and its natural or acid salts are called pectate	Conrad 1930
3	Protopectin	Water-insoluble parts of pectic substances, mostly present in unripe fruit and it degrading by protopectinases	Sharma and Satyanarayana 2004
4	Pectin	75% of carboxyl groups of galacturonic acid units are esterified with methanol	Thakur *et al.,* 1997

FUNGAL PECTINASES

Pectinases are defined as a group of enzymes that hydrolyse pectic substances, mostly present in microorganisms and higher plants (Sharma and Satyanarayana, 2004; Jayani *et al*., 2005). The majority of commercial pectinases are obtained from fungi.

Diversity of fungal pectinases and their categorization

There is a great diversity of fungal pectinases, which can be classified into several categories based on their mode of action, substrate specificity and amino acid sequence (Chen *et al.,* 2015). On the basis of their pH optima for activity, they are categorized into acidic and alkaline pectinases.

On the basis of pH optima for activity:

Acidic pectinases

The pectinases with pH optima below 7 are called acidic pectinases. These are primarily produced by fungal strains such as *Aspergillus niger, A. flavus, A. oryzae, Penicillium italicum, Trichoderma viride, T. reesei* and others. These are most widely exploited for the large-scale production of acidic pectinases (Haile and Ayele, 2022).

Alkaline pectinases

Optimum pH for the activity of alkaline pectinases is above 7. Alkaline pectinases are generally produced by bacteria, but are also produced by some filamentous fungi and yeasts. Alkalophilic bacteria, such as *Bacillus* spp. are primarily used for the commercial production alkaline pectinases (Hamdy *et al*., 2005). On the other hand, fungal spp. such as *Fusarium, Rhizopus, Penicillium, Aspergillus* etc., are also reported as potent alkaline pectinase producers (Thakur *et al.,* 2021).

On the basis of mode of action:

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Esterases

Pectinesterases (PMGE, EC 3.1.1.11)/ Pectin pectylhydrolase/Polymethylgalacturonate esterases

Pectin esterases are enzymes that specifically remove the methyl ester group from pectic substances, resulting in the formation of pectic acid. This process involves the formation of an intermediate acyl-enzyme complex and the release of methanol. Eventually, the acyl-enzyme complex undergoes diacylation, leading to the restoration of both the enzyme and pectic acid (Oumer *et al.,* 2017). The enzyme tends to target the methyl ester groups that are positioned adjacent to free carboxyl groups along the length of the polymer in a sequential manner (Patidar *et al.,* 2018). Some pectin esterases only act on the reducing end of the chain, while others target the non-reducing end. The specificity for different substrates can vary depending on the source of the enzyme and the reaction conditions **(Figure 5).** Pectin esterases are widely distributed in nature and can be found in microorganisms, plants and animals; fungal pectin esterases are of peculiar interest (Rebello *et al.,* 2018). Pectin esterases derived from microbes have a broad pH range for activity, functioning effectively at pH between 4.5 and 9.0. Fungal pectin esterases tend to have a lower optimal pH compared to bacterial pectin esterases (Patidar *et al.,* 2016). Notably, alkaline and acidic pectin esterases exhibit different de-esterification patterns, leading to the formation of pectin with varied properties (Kant *et al.,* 2013). Alkaline pectin esterases produce de-esterified pectin that forms weak gels with calcium ions, while the product of acid pectin esterase form stronger gels with calcium ions.

In a study conducted by Jenkins *et al.* (2001), the crystalline structure of pectin esterase was examined. The molecule had a molecular mass of 36.91kDa and consisted of 342 residues. The molecule had an α content of 6.43 and a β content of 34.80. The structure of the molecule included four sheets and two β-hairpins with one disulphide bond.

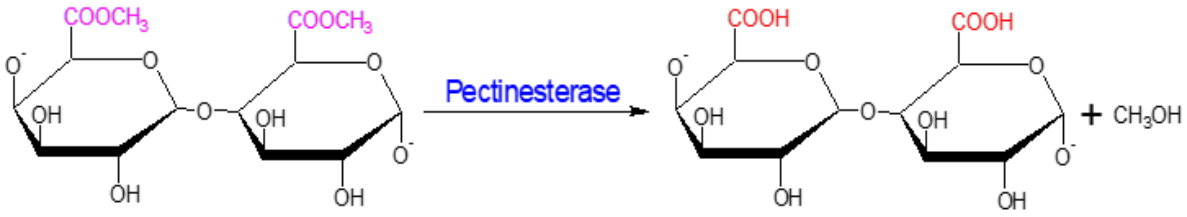

Figure 5: Reaction mechanism of pectinesterases.

Pectin esterases have an optimum temperature ranging between 25 and 55°C. Pectin esterases mostly originate from plants, bacteria and pathogenic fungi belonging to the class 8 of carbohydrate esterases **(Figure 6).** PMEs are biologically active in monomeric form and bear a molecular mass within the range of 25-45kDa. The molecular mass of pectin esterases of *Aspergillus oryzae* is about 34kDa and 400kDa that of *Clostridiummu ltifermentans* (Rebello *et al.*, 2018). The precise mechanism of action of pectinesterases (PEs) is still debated, but it is generally believed that there are three different modes of action depending on the source of the enzyme. Fungal pectinesterases (PEs) primarily operate through the multiple-chain mechanism, while bacterial and plant PEs typically exhibit single-chain and multiple-chain mechanisms (Singh *et al.*, 2019).

One of the commonly used methods to measure the activity of pectinesterases is the titrimetric method, where the amount of carboxylic acid produced is measured by the amount of alkali used to neutralize the reaction mixture (Chawafambira, 2021).

Another method involves measuring the amount of methanol produced through distillation and oxidation to formaldehyde. Wood and Siddiqui (1971), suggested a simplified spectrophotometric method that eliminates the need for methanol distillation. The most accurate and sensitive method involves converting methanol to methyl nitrite and measuring it through HPLC.

Polygalacturonases (PG, EC 3.2.1.15) / Poly (1,4-α-D-galactosiduronate) glycanohydrolase/Endo -polygalacturonase

Polygalacturonases are enzymes that break down pectin by adding water molecules to the glycosidic bonds. These enzymes are typically classified as acidic pectinases and specifically target the alpha-1,4-glycosidic linkages in pectic acid or polygalacturonic acid (Patidar *et al.*, 2018). Although there are some reports of alkaline endo-polygalacturonases, they are relatively rare. Most of the fungal pectinases produced by *Aspergillus japonicus, Rhizopus stolonifer, Alternaria mali, Fusarium oxysporum, Neurospora crassa, Penicillium italicum ACIM F-152* and many others have optimal pH range between 3.0 and 6.0 (Jayani *et al.*, 2005).

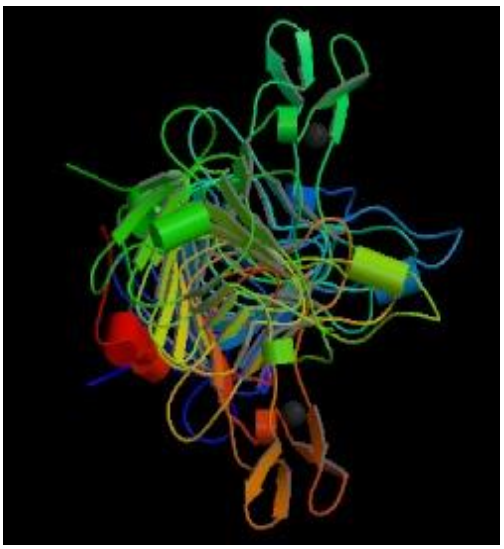

Figure 6: Crystalline structure of pectinesterase of *Erwinia chrysanthemi* (Jenkins *et al.*, 2001).

Lyases

The Lyases (transeliminases) form a group of pectinolytic enzymes that catalyze the breakdown of either pectic acid (polygalacturonate lyases) or pectin (polymethylgalacturonate lyases) by β-elimination reaction. Two types of lyases are known.

Endopolygalacturonate lyases (Endo-PGL, EC 4.2.2.2)/ Poly (1,4-α-D- galactosidu-ronate) endolyase/Endopolygal-acturonate lyase (endopectate lyase)

They are a group of hydrolytic enzymes, which randomly cleave α-1,4-glycosidic bonds by β-elimination in pectates and pectic acid, resulting in a rapid decrease in viscosity compared to the number of bonds broken **(Figure 7).** The pectate lyases are isolated from *Aspergillus flavus, Fusarium oxysporum* and others. It degrades the substrates in endo-acting manner **(Figure 8)** well-known enzymes come from the *Erwinia* and *Bacillus* genera and are known to cause soft rot symptoms in plants, as reported by Kabli *et al.* (2007). Endo-polygalacturonate lyases typically adopt a beta-helix fold, which consists of repeated beta- strands connected by loops, forming a twisted and elongated structure.

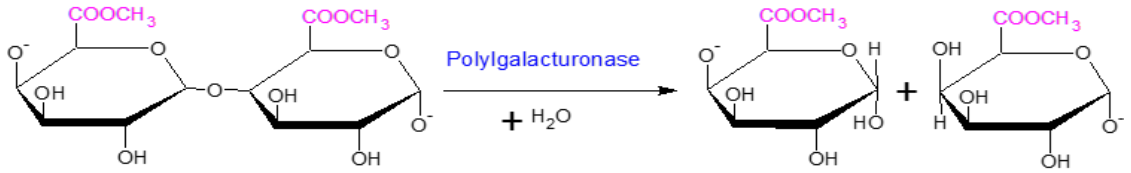

Figure 7: Reaction mechanism of polygalacturonase.

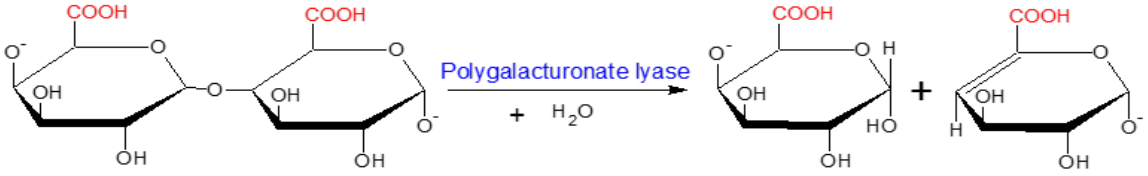

Figure 8: Reaction mechanism of polygalacturonate lyase.

Endo-polygalacturonate lyases are enzymes mostly produced by microorganisms. The active site of endo-polygalacturonate lyases contains amino acid residues critical for substrate binding and catalysis. These residues are usually located within a cleft or pocket in the enzyme structure. However, endo-polygalacturonate lyases can also be found in other microorganisms like *Aspergillus*, *Fusarium* and *Streptomyces* and others. The temperature optima for endo-polygalacturonate lyases can vary depending on the enzyme source. Most endo-polygalacturonate lyases are active within a temperature range of 30-50°C (Zheng *et al.*, 2021).

Exopolygalacturonate lyases (Exo-PGL, EC 4.2.2.9)/ Poly (1, 4-α-D-galactosiduronate) exolyase/ Exopolygalacturonate lyase (exopectate lyase)

Exo-polygalacturonates primarily act on pectates instead of pectins and they do not affect polymethyl-galacturonate-methylglycoside. They hydrolyze oligo-galacturonic acid from the reducing end of the substrate chain and the smallest substrate that they can degrade is a trimer. Both exo and endo-polygalacturonases have an average molecular mass of 38-65 kDa, the enzymes are reported from various microbial sources (Jayani *et al.*, 2005). They are most active in the pH range of 8.0 to 9.5. Exo-PGLs also have about similar thermostability as endo-PGL. Most exo-polygalacturonate lyases are active within a temperature range of 30-50°C (Shevchik *et al.*, 1999). When working together on pectin, the complex of polygalacturonate lyase and pectin esterase allows the pectin chain to move directly from the esterase site to the polygalacturonate lyase site without dissociation or rebinding (Wong and Ng, 2011). Heating can cause the polygalacturonate lyase to be released from the pectin esterase. Exo-polygalacturonate lyases are stabilized by $Ca^{2+}$and Mn^{2+}, but not by Mg^{2+}or Zn^{2+}

Poly-methylgalacturonate lyases (PMGL)

These are all endo-acting enzymes that catalyze the β-elimination between the fourth and fifth carbons of pectin at the non-reducing end, causing a quick

drop in viscosity **(Figure 9, 10).** They are mostly of fungal origin and can degrade pectin directly, although they prefer highly esterified pectin as a substrate (Ankita *et al.,* 2000). PMGL enzymes are expected to exhibit a degree of thermal stability. The optimum temperature stability range may vary among bacterial and fungal enzymes, they are generally active within a moderate to high temperature range which is from 30 °C to 70 °C or higher. However, further studies specific to PMGLs are necessary to determine their optimal temperature range and thermal stability. They are most active when the pH is between 5.0 and 9.0. They do not require metal ions for their activity. Depending on the pH and degree of esterification of pectin, a few authors have reported the need for Ca^{+2} and other cations. The optimum pH and temperature were 5.0 and 55 °C (Patidar *et al.,* 2017).

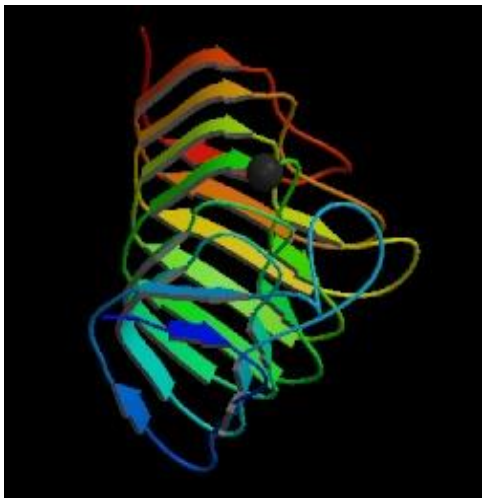

Figure 9: Crystal structure of polygalacturonate lyase (Ankita *et al.,* 2000).

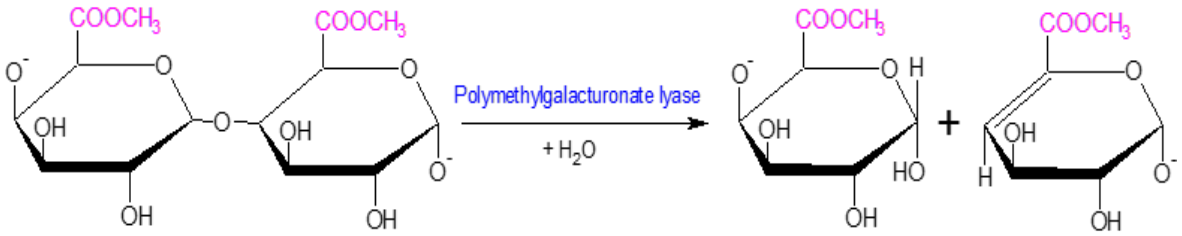

Figure 10: Reaction mechanism of poly-methylgalacturonate lyase.

As the chain length of the substrate decreases, the activity of polymethyl galacturonate lyases falls rapidly. *Aspergillus fonsecaeus* enzyme degrades smallest substrates, tetramethyl tetra galacturonate. While that of *Aspergillus niger* degrades trimethyl-tri-galacturonate. They are further divided into endo and exo-polymethyl galacturonate lyases based on their cleavage patterns. By trans-elimination, endo-polymethyl galacturonate lyases cause random cleavage in pectin molecules. While, exo-polymethyl galacturonate lyases cause sequential cleavage in pectin by a trans-elimination process.

Protopectinases (EC 3.2.1.99)

Protopectinases are enzymes that convert insoluble protopectin into highly polymerized water-soluble pectin. They are classified as A- and B-type based on their mode of action (Tapre and Jain, 2014). A-type protopectinases react with the smooth regions of protopectin, which are composed of partially methoxylated galacturonic acid residues, while B-type protopectinases react with the hairy regions consisting of rhamnogalacturonans and neutral sugar side chains **(Figure 11)**.

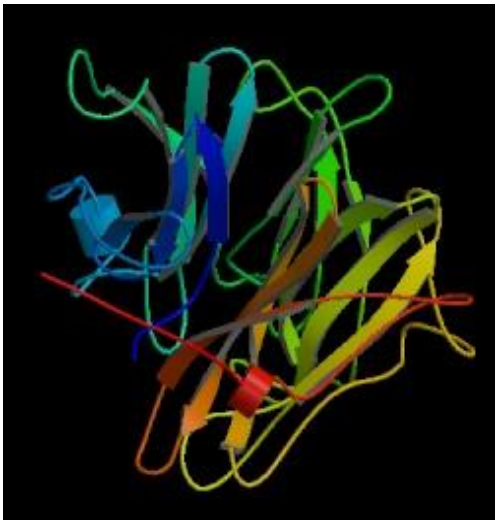

Figure 11: Crystalline structure of protopectinase C (Arabinan endo-1,5-alpha-L-arabinosidase) (Nurizzo *et al.,* 2002).

As a result, B-type protopectinases are considered as glycan hydrolases such as arabinases and galactanases (Nurizzo *et al.,* 2002). Some fungal protopectinases have been reported to have molecular masses in the range of 30-40 kDa, while others can be larger, with molecular mass exceeding 100 kDa (Nurizzo *et al.,* 2002). Kabli (2007) isolated protopectinases showed that the enzyme was fairly stable at 50ºC since it retained 65% of activity by exposure to this temperature for 30 min at pH 5.0. The optimal temperature for most of the fugal the protopectinases lies between 50 to 55°C (Silva *et al.,* 2002). Protopectinases have a similar and positive effect of Ca^{+2} and Mg^{+2} ions and exhibit maximum enzyme activity in their presence, whereas it lost most of the activity in the presence of Cu^{+2} and Hg^{+2} ions (Sharma *et al.,* 2023).

METHODS FOR PRODUCTION OF PECTINASES

Production of enzyme on large scale is a necessity for their utilization in the industries (Kumar *et al.,* 2012). Various fermentation processes are developed for their production which can be classified under the following two categories:

Solid-State Fermentation: In this, microorganisms are grown on a solid agro-residue, such as wheat bran or rice husk, which provides the necessary nutrients for growth. The substrate is inoculated with pectinase-producing microorganism and the mixture is incubated under controlled conditions (Garminha *et al.,* 2008). Several fungal strains such as *A. niger, A. awamori, Trichoderma reesei, Penicillium* etc. have been used extensively for the solid-state fermentation (SSF) for the production of fungal pectinases (Samreen *et al.,* 2019).

Submerged Fermentation: This method involves the cultivation of microorganisms in a liquid medium. For submerged production of pectinases, fungal species such as *Rhizopus oryzae, R. stolonifer, Trichoderma reesei, Penicillium chrysogenum P. digitatum, Aspergillus oryzae, A. niger etc.,* are most common because these can grow vigorously in submerged condition (Abdullah *et al.,* 2018). Corn cobs, citrus waste, apple pomace, corn steep liquor etc. are the most preferable substrates for submerged fermentation. During SmF, apple pomace substrate is best suited substrates for the exponential growth of *A. niger* and *T. reesei* (Mahmoodi *et al.,* 2017).

FACTORS THAT INFLUENCE THE PRODUCTION OF PECTINASES

The production can be influenced by various physical and chemical factors, including the microorganism used, nutrients, pH, temperature, oxygenation, inoculum size, fermentation time and substrate used etc. Common parameters influencing pectinase production are

Substrates

The selection of substrate to grow the producer fungal strain is an important aspect. Pectinases are generally produced in response to the presence of pectin or other pectin-containing substrates (Singh

et al., 2017). Different substrates can have different levels of pectin and the type and amount of substrate used can affect the yield of pectinases because the producer fungal strains may have variation in utilization of the substrate molecules (Samreen *et al.,* 2019). Here are some ways in which the substrate can influence pectinase production.

Substrate type

Different substrates contain varying amounts and types of pectin, which can affect pectinase production. For example, citrus peel waste is a good source of pectin and has been shown to enhance pectinase production by *Aspergillus* and *Fusa*rium spp. (Panchami and Gunasekaran, 2015). Different agro-wastes, such as sawdust, pineapple peel, orange peels, sugarcane pulps and wheat bran, as the sole carbon source reported the highest pectinase activity by *Aspergillus niger, A. fumigatus, A. giganteus, A. terreus* and *Penicillium chrysogenum* (Okafor *et al.,* 2010).

Substrate concentration

The concentration of the substrate affects pectinase production. A low concentration of pectin may not provide enough induction for producing pectinases, whereas a high concentration of pectin may result in product inhibition (Mohandas *et al.,* 2018). Therefore, the optimal concentration of the substrate needs to be determined for efficient pectinase production. The production with different concentration of carbon sources (0.5%, 1%, 1.5%, 2%, 2.5%, and 3% w/v) resulted maximum production of polygalacturonase at 1% concentration (Darah *et al.,* 2013).

Particle size

The particle size of the substrate also affects pectinase production. Smaller particle sizes can improve oxygen transfer in SSF. The increased surface area allows better aeration and oxygen penetration into the substrate, facilitating fungal growth and enzyme production. This improved contact leads to increased enzymatic degradation of the pectin, resulting in higher pectinase production (Rehman *et al.,* 2015). In SmF, increased surface area allows for better contact between the fungal biomass and the substrate, potentially leading to improved enzymatic activity and higher pectinase production (Poondla *et al.,* 2016).

Pre-treatment of substrate

The effect of substrate pre-treatment on pectinase production from *Aspergillus niger* vary depending on the method used like thermal, chemical, enzymatic and biological pre-treatments are commonly employed (Jalil *et al.,* 2023). Thermal pre-treatment can enhance pectinase production by breaking down complex polysaccharide structures. Chemical pre-treatment solubilizes pectin, increasing its availability for enzymatic degradation (Belkheiri *et al.,* 2021). Enzymatic pre-treatments with cellulases or hemi-cellulases degrade other components, improving pectin accessibility whereas biological pre-treatments, involves microbial cultures which facilitates the breakdown of substrate (Rebello et al., 2017).

Substrate availability

The availability of the substrate affects pectinase production. Microorganisms require a continuous supply of nutrients for efficient growth and pectinase production (Minten *et al.,* 2014). Therefore, the fermentation process should be designed to ensure a constant supply of substrate.

pH

The pH of the growth medium has a significant influence on the production of pectinases. The pectinase retained 60-90% activity in the range between 6.2 and 9.2 (Khatri *et al.,* 2015).

Optimal pH range

Each microorganism has an optimal pH range for its growth and enzyme production. For most pectinases, the optimal pH range lies between 4.5 and 5.5. Maciel *et al.* (2011) reported the wide pH range (3.5-11.0) for enzyme production by *Aspergillus niger*. Therefore, maintaining the pH within this range during fermentation affects pectinase production (Sharma and Satyanarayana, 2006; Suresh, 2010).

Enzyme stability

The stability of pectinases is influenced by pH. Most pectinases are stable at acidic pH values, but their stability decreases as the pH becomes more alkaline (Irshad *et al.,* 2014). Fungal alkaline pectinases showed high stability and active at alkaline pH. Therefore, maintaining the pH within the optimal range can help in maintenance of

enzyme stability and activity. The partially purified pectinase from *Aspergillus niger* strain MCAS2 was significantly active over a pH range from 6.2 to 9.2 with an optimum of 8.2 (Khatri *et al.,* 2015). Exo-polygalacturonase produced by *Rhizomucor pusillus* A13.36 possessed optimal activity at pH 4.0 but found stable in the pH range of 3.5 to 9.5 (Trindade *et al.,* 2016).

pH shift

A sudden shift in pH can negatively impact pectinase production. For example, if the pH of the medium becomes too acidic or too alkaline, the microorganisms may not be able to grow and produce pectinases efficiently (Joshi *et al.,* 2008). Enshasy *et al.* (2018) noted that a pH shift from 5.5 to 3.6 decreased the cell growth from 3.28 g/L to 2.38 g/L during the process optimization for *A. niger* in submerged cultivation system.

pH control

pH control during fermentation is important for efficient pectinase production. The pH can be controlled using a buffer system or by adding acid or base to the medium (Ahmed *et al.,* 2016). However, the method of pH control used can affect pectinase production. Selection of buffer is also an important parameter. The most frequently used buffers include phosphate, citrate or acetate salts. The choice of buffer should be based on the desired pH range and the fungal strain being used. *A. niger* IBT-7 strain produced optimal pectinases production when sodium acetate buffer was used (Abdullah *et al.,* 2018).

Temperature

Temperature is another important factor that can influence pectinase production. Here are some ways in which temperature can affect pectinase production:

Optimal temperature range

Each microorganism has an optimal temperature range for growth and enzyme production. Cheng *et al.,* (2016), evaluated the thermal stability and activity for pectinases, produced by *Aspergillus* spp. and found the optimal temperature range between 30°C and 50°C. Pectinase produced by *Aspergillus* species have been reported to get inactivated due to denaturation at temperature above 50°C (Khatri *et al.,* 2015).

Enzyme stability

The stability of pectinases is influenced by temperature. Most pectinases are stable at moderate temperatures but can be denatured at high temperatures (Biz *et al.,* 2014). Therefore, maintaining the temperature within the optimal range helps maintaining enzyme stability and activity.

Temperature shift

A sudden shift in temperature negatively impacts pectinase production (Jalil and Ibrahim, 2021). Exposing the microorganisms to extreme temperature scan led to cell death or denaturation of pectinases. In case of fungal pectinase production Joshi *et al.* (2008) reported that there was a constant increase in production up to 50°C and after that it started declining slowly up to 65°C and then a sharp decline up to 80°C.

Temperature control

Temperature control during fermentation is important for efficient pectinase production. Temperature can be controlled using a heating or cooling system (Chaudhary *et al.,* 2020). Use of heat exchangers to regulate temperature by exchanging heat between the fermentation medium and a heat transfer fluid is very common method of temperature control. Cooling water or refrigerated liquid can be circulated through the heat exchangers to lower the temperature, while heated liquid or steam can be used to raise the temperature. After application of the temperature control system, Begum *et al.* (2020) recorded 2% excess production of all pectic enzymes in submerged fermentation by *Aspergillus niger* and *Aspergillus flavus.*

Agitation

Agitation or mixing of the fermentation medium is known to influence the production of pectinases in several ways:

Oxygen supply

Agitation affects oxygen supply to the microorganisms, which is essential for their growth and metabolism. Improved oxygen supply can lead

to increased pectinase production. Adequate oxygen supply is crucial for the growth and metabolism of microorganisms (Sharma and Rishishwar, 2015).

Nutrient availability

Agitation can help in distribution of nutrients evenly throughout the medium, ensuring that all growing cells have access to sufficient nutrients. Ibrahim (2015) evaluated the enzyme activity of pectinases produced by *Aspergillus niger* HFD5A-1strain from static to 50, 100, 150, 200 and 250 rpm and reported optimal pectinase production at 150 rpm (1.559 U/mL). Jalil *et al.* (2023), achieved highest pectinases activity produced at 150 rpm, and the fungal growth was 2.49 ± 0.02 g/L for *Aspergillus niger* LFP-1. Maximum production of pectinases by *A. niger* MTCC281 was attained at 170 rpm. With an increase in the rpm level from 100 rpm with a gradation of 20 there has been an increase in pectinases production and maximum activity of 5.04 U/ml was recorded (Palaniyappan *et al.,* 2009).

Shear stress

High agitation rates result in high shear stress on the fungal mycelia, which can damage their cell walls and reduce pectinase production. Therefore, optimal agitation rates need to be determined to avoid excessive shear stress (Jalil *et al.,* 2023).

Mixing efficiency

The efficiency of mixing can also affect pectinase production. Poor mixing can lead to uneven distribution of nutrients and oxygen, resulting in a decline in pectinase production. The influence of agitation speed was found to lay a great impact on the morphology of *A. niger* HFD5A-1. At static condition, the mycelial mat was discovered on the top of the cultivation broth and sporulation occurred all over the fungal mycelial mat (Ibrahim, 2015).

Foam Accumulation

High agitation rates can also lead to excessive foam production. Excessive foam formation leads to increased viscosity, reduced mass transfer and decreased oxygen availability that negatively affects microbial growth and enzyme production. Additionally, foam can cause mechanical stress on the fungal mycelium, leading to cell damage and reduction in enzyme production (Ogueji *et al.,* 2017).

CHARACTERISTICS OF FUNGAL PECTINASES

Enzymatic breakdown of the substrates and the rate of enzymatic reactions depend upon the characteristics of the enzyme like pH, temperature, incubation time, metal ions, substrate concentration etc. (Rani *et al.,* 2012).

Km value

The Km value represents the substrate concentration at which the enzyme-catalyzed reaction rate is half of its maximum velocity (Vmax). A lower Km value indicates a higher affinity of the enzyme on its substrate, while a high Km value indicates a lower affinity. Km value for fungal pectinases can vary according to the condition of analysis. The kinetics of pectinase produced by *Aspergillus niger* in submerged fermentation was found that, the Km value for the enzyme was 0.125 mM under optimal conditions of pH 4.5 and temperature 50°C (Alqahtani *et al.,* 2022). Hao *et al.* (2022) analyzed the kinetics of purified endo-polygalacturonase of *Penicillium griseoroseum* and found that the Km value for the enzyme was 1.01 mM at pH 4.0 and 50°C. A study that investigated the kinetics of purified pectinase of *Aspergillus flavus* found that the Km value for the enzyme was 28.9 mM at pH 5.5 and 45°C (Gophanea *et al.,* 2016). Fungal pectinases with low Km values (ranging from 0.01 to 0.3 mM) have been reported to exhibit high catalytic efficiency and activity towards pectin.

Effect of metal ions on pectinase activity

Metallic ions display a significant role in modulating the activity of fungal pectinases (Jalil *et al.,* 2023). The effect of metal ions on pectinase activity depends on their charge and concentration. The impact of metal ions on pectinases activity is listed in **Table 2**. The presence of Na^{+}, Mn^{+}, Cu^{2+} and Zn^{2+} led to a significant increase on enzyme activity, particularly polygalacturonase (PG). Conversely, the presence of Fe^{2+} and Mg^{2+} caused a significant decline in PG activity (Dranca and Oroian, 2018). For pectin lyase, the activity was significantly enhanced by Fe^{2+}, Mn^{+} and Zn^{2+}, while it was significantly reduced by Cu^{+}, Mg^{2+} and Na^{+}. Hamdy (2005) observed that ions of Mg^{++}, Na^{+} and K^{+} showed a stimulatory effect, whereas

Zn^{2+}, Co^{2+}, Mn^{2+} and Hg^{2+} showed inhibitory effects on pectin lyase of *Rhizopus oryzae*. Daniel *et al.* (2014) also observed that manganese ions stimulated pectinlyases. However, Yadav *et al.* (2013) reported that Ca^{2+}, Cu^{2+}, Fe^{3+}, Mn^{+}, Mo^{2-}, Ba^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+} and Ag^{+} did not stimulate pectin lyase activity.

Effect of pH on pectinase activity

The pH optima of pectinase is likely due to the fact that the enzyme's active sites have variable charge at different pH (Joshi *et al.*, 2008). At other pH values, the active site may be too charged or too uncharged to bind to its substrate, resulting in decreased enzyme activity. Additionally, the activity of pectinases can also be affected by the pH of the substrate. Kumar *et al.* (2012) found that the activity of pectinase of *Aspergillus niger* was low on pectin substrates at a higher pH than the enzyme's optimal pH. This could be due to the fact that the pectin substrate is also charged and the interaction between the charged pectin substrate and the charged enzyme active site can disrupt the enzyme's ability to bind to its substrate and catalyze the reaction.

The greatest activity of pectinases are recorded at acidic pH values, with an optimum pH of around 4.0. Joshi *et al.* (2015), found that the optimal pH for pectinase activity of *Aspergillus niger* was 3.5. Other studies have found that the optimal pH for pectinase activity from other sources, such as *Bacillus subtilis* and *Trichoderma viride*, is around 5.0.

Effect of temperature on pectinase activity

Thermostability of an enzyme is the ability to resist thermal unfolding in the absence of substrates. Several studies have investigated the thermostability of pectinases. Hasunuma *et al.* (2003), found that endo-PG produced by *A. niger* T0005007-2 lost about 40% of its enzymatic activity after exposure to 50°C for 60 min. Ortega *et al.* (2004) reported similar results for a commercial pectinase preparation called CCM. The CCM preparation retained 57% of activity after exposure to 50°C for 60 min. However, the commercial fungal pectinases Rapidase C80 and Pectinex 3X were much less stable at this temperature. Only 5.0 to 10.0% of activity was retained after 60 min at 50°C. Cordeiro and Martins (2009) found that the optimum temperature for the activity of Polygalacturonase produced by *Bacillus* sp. was 70°C. The enzyme retained 90% of activity after exposure to 70°C for 120 min. Fungal pectinases are most stable at 30 - 50°C (Sandri and Silveira, 2018). However, there is some variation in thermostability depending on the source of the enzyme. The decrease in enzyme activity at higher temperatures may be due to enzyme denaturation. Denaturation is a process in which the protein's structure is disrupted, which can lead to a loss of activity. The higher the temperature, the faster the rate of denaturation. In case of fungal pectinases, the optimum temperature for activity is 35°C (Trindade *et al.*, 2016). At this temperature, the enzyme is most stable and has the highest activity. If the temperature is increased beyond 35°C, the enzyme begins to denature and loses its activity. This is why the fungal pectinase activity decreased as the temperature increased beyond 35°C.

POTENTIAL INDUSTRIAL APPLICATIONS OF FUNGAL PECTINASES

Global research is focusing on improving the activity of pectinases and expand their applications. Pectinases are in high demand worldwide and their applications vary depending on the physical conditions. Pectinases have been used in conventional industrial processes such as textile, plant fiber processing, tea, coffee, oil extraction and industrial wastewater treatment.

Food biotechnology

Pectinases offer numerous advantages, including increased efficiency, milder processing conditions and improved product quality. They are widely employed in various food processing and manufacturing processes, contributing to the development of innovative and improved food products (Atta and Larrea, 2022). It is used in processes such as juice extraction, fruit juice clarification, winemaking, coffee and tea fermentation, refining vegetable fibers, curing coffee, cocoa and tobacco, canning orange segments and extracting sugar from date fruits etc. Pectinases are widely used in the fruit industry for juice extraction. They help to reduce the viscosity and enhance clarity of juice. High pectin content of fruits makes their juice cloudy, which creates issues for clear juice processing and marketability (Garg *et al.*, 2016). As traditional juice extraction methods are energy-intensive and unappealing, pectinase is used as a pre-treatment before clarification to prevent pectin-protein flocculation and reduce viscosity, the early use of pectinase in

fruit juice to improve permeation flux in microfiltration, ultrafiltration and reverse osmosis processes (Kashyap *et al.,* 2001). Pectinase has been found to effectively reduce juice viscosity, increase yields and contribute to the release of phenolic compounds from fruit skin.

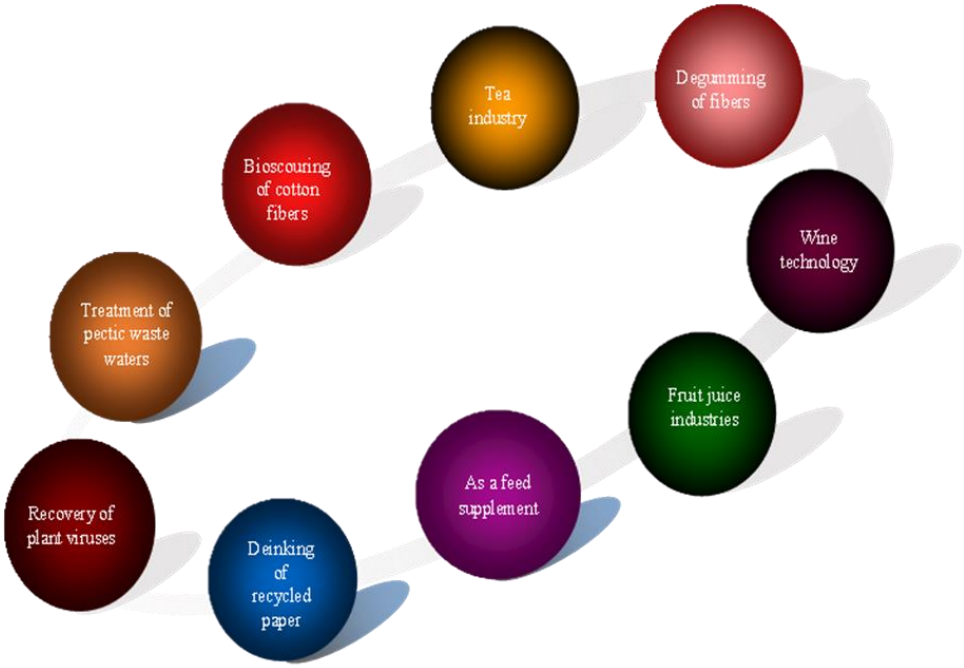

Figure 12: Application of pectinases

Pectinases are also used in winemaking to quicken the maceration process, increase the amount of juice extracted, hastening filtration and enhancing the flavor and color of wine. The fruits are macerated with pectinases before the alcoholic fermentation is introduced to improve wine quality. By adding pectic enzymes during the crushing of fruits, the juice yield is increased and pressing time is reduced (Praveen and Suneetha, 2015). Additionally, the filtration process is improved, resulting in clearer juice and the stability and color of red wines.

In tea and coffee fermentation, use of pectinases in processing is important for improving the quality and appearance of the final product. Instant tea powder is widely used in the preparation of tea, as it is made from tea leaves and contains a high concentration of pectin (Oumer *et al.,* 2017). However, the use of this powder can lead to the formation of foam on the surface of the tea, due to the high pectin content. To improve the quality and appearance of the tea, polygalacturonase is used in the tea-making process. It helps to break down the pectins in the instant tea powder, thereby reducing the foam-forming property of the tea. This can result in a change in the color of tea and can increase its market value (Patidar *et al.,* 2016).

Pectinase is also utilized in the coffee fermentation process to improve the quality of the coffee beans. The coffee beans are surrounded by a hardcover called mucilage, which has viscous and gelatinous properties that are undesirable for making coffee. In the alkaline process, pectinase is used to break down the pectin in the mucilage coat, making it easier to remove from the coffee beans before roasting (Haile and Ayele, 2022). During the fermentation of coffee, pectinolytic microbes are also used to improve the quality of coffee beans and to remove the mucilage coat. Overall, the use of pectinases in the coffee and tea production processes can improve the quality of the final products and lead to better flavor and texture.

Textile industry

The use of pectinases in the textile industry offers several advantages. They provide an eco-friendly alternative compared to traditional chemical processes, as pectinases are derived from natural sources such as microorganisms and plants.

Pectinases also offer versatility in their application, as they can be used on various types of fibers, including cotton, linen and blends (Rajendran *et al.,* 2011). Furthermore, they contribute to the production of high-quality textiles with improved performance, comfort and aesthetic appeal. Sizing agents such as starch are applied to the fibers to improve their handling and weaving properties. These sizing agents need to be removed before further processing.

Table 2: Physiochemical and Kinetic Properties of Pectinases

Enzyme	Molecular weight (kDa)	Michaelis constant (mg/mL)	Optimum temperature (ºC)	Optimum pH	Temperature stability (°C)	pH stability	Enhanced	Reduced	Effects of ions on enzyme stability References
Endo PG	38	1.27	65	5	50	2.2-7	Na, K, Ca, Mg, Fe	Mn, Co, Zn, Cu	Cheng *et al.*, 2016
	40	0.19	40	4.5	40	3.5-5.5	K	Ca, Mn, Mg, Cu	Tan *et al.*, 2020
	42	-	35	6	30-40	4-9	Mg	Na, Ca, Fe, Ba, Zn, Cu	Sassi *et al.*, 2016
	71	1.25	55	3.5	55	2-7	Mn, Mg	Ca, K, Na, Ni	Yang *et al.*, 2011
	60	2.45	70	4.5	50-60	6-8	-	Ca, Fe, K, Mg	Carli *et al.*, 2019
	106	0.083	45	4.8	25	3.5-5.5	Mg, Cu	Ca, K, Al, Zn	Kant *et al.*, 2013
	29	2.08	40	5	10-40	5-11	Co, K, Cu	Ag, Ca, Hg	Anand *et al.*, 2017
Exo PG	102	2.56	50	5	50	3-5	Mg	Ca, Na, K, Cu	Pognonceli *et al.*, 2019
	75.28	5.44	60	5	30-50	3-11	Cu, Zn, Na, K, Mg	Hg, Ba	Lu *et al.*, 2016
	50	0.31	72	5.2	70	-	-	Co, Ni, Ca, Mg	Chen *et al.*, 2014
	60	0.29	50	10.5	-	-	Ca, Mn	Pb, Sn, Cu	Sharma and Satyanarayana 2020
	31	3.87	50	7.5	50-60	7-9.5	Ca, K, Mg, Na	Co, Cu, Mn, Zn	Hamdy 2005
	23.3	5.2	40	8	-	-	Mg, Ca, Fe, Na	K, Co	Porturcu *et al.*, 2017
Pectin lyase	50	1.7	55	8	10-40	3-11	Mn, Ca	Hg, Ag, Zn	Yadav *et al.*, 2013
	40	-	50	4.5	30-40	3-5	Na, Mg, Zn, Ni	K, Ca, Cu	He *et al.*, 2018
	31	1.75	40	9	10-50	5-7	Ca, K, Na, Zn, Ag	-	Yadav *et al*., 2017
	44	1.78	55	9.8	-	4-10	Ca, K	Ba, Mn	Wang *et al.*, 2014
	38	1.64	50	8	40-60	5-10	Ca	Ni, Mn, Zn, Cu	Guan *et al.*, 2020
Pectate lyase	41	-	50	4	40	6-9	Al, Mg	Fe, Ba, Cu	Yang *et al.*, 2020
	43.1	0.312	50	9.5	30-60	5-11	Ca, Mg, Mn, Ba	Cu, EDTA	Zhou *et al.*, 2017
	34	0.45	60	10.5	70-90	7.5-12	Ca, Mn, Ba, Mg	Hg, Cu, Sn, K	Saoudi *et al.*, 2015
	141	1.3	30	6.5	-	-	Ca, Na, Mg, K	Pb, Hg	Arotupin *et al.*, 2008
Pectin methyl esterase	33	0.008	60	9	40-60	-	Na	-	Dixit *et al.*, 2013
	27	0.22	60	7	30-60	4-10	Na, K, Mg, Ca	Zn, Ni, Cu, Fe	Kotnala *et al.*, 2018
	37	3.27	45	3.8	20-30	2-6	-	-	Zhang *et al.*, 2018

Pectinases are used in desizing to break down and removal of pectin-based sizing agents effectively (Nawaj *et al.,* 2018). This process improves the fabric's absorbency, prepares it for dyeing or printing and enhances its overall quality. Pectinases are employed in bio-polishing treatments to improve the surface appearance and handle of fabrics (Kumar *et al.,* 2021). When applied to cotton or other cellulosic fibers, pectinases selectively remove the protruding fiber ends, pills and fuzz, resulting in a smoother and softer fabric. This process enhances the fabric's aesthetic appeal, reduces the potential for pilling and improves its comfort and drapability (Oumer, 2017).

Pectinases also play an important role in dyeing processes by enhancing the penetration and fixation of dyes on textile fibers. Pectin substances naturally present in fibers can hinder dye diffusion and bonding (Kashyap *et al.,* 2001). By removing or modifying these pectic substances, pectinases facilitate better dye penetration into the fibers and improve color fastness, resulting in more vibrant and even dyeing. Pectinases are used in fabric finishing treatments to enhance various properties of the textiles.Pectinases can be used to modify the surface characteristics of fabrics, leading to improved moisture absorption and wicking properties (Nighojkar *et al.,* 2019). Additionally, pectinases can be employed in wrinkle-resistant finishing processes, where they help in the controlled relaxation of fabric fibers, resulting in reduced wrinkling and improved fabric appearance blends (Rajendran *et al.,* 2011). Specific enzyme formulations and application conditions may vary depending on the fabric type and desired effects, making careful consideration of the specific requirements and characteristics of the textiles being processed,

Paper production

Enzymes are becoming increasingly popular in the paper industry because they transform the substrate of interest in the presence of other chemically related molecules. Pectinase is used to overcome retention problems in mechanical pulps which have been bleached with hydrogen peroxide (Bajpai, 2018). The papermaking process involves continuous filtering a slurry of fibers, fiber fragments (fines) and inorganic filler particles (clay or $CaCO_3$) into sheets. Water must be removed regularly (Verma *et al.,* 2017). A filter cloth with pores is used for this, allowing the fiber fragments and filler particles to flow through. Some drainage mechanisms are used to keep the "fines" and "filler" in the paper sheets in place, allowing for faster water drainage. Various cationic polymers with different architectures are commonly used as retention aids (Liu *et al.,* 2017).

Bleaching pulps with alkaline peroxide solubilize the polysaccharides present in the pulp, the most notable things are pectins or polygalacturonic acid, which are interfering compounds. Polygalacturonic acids have a tendency to form gels with cationic polymers (cationic demand). Cationic demand is highly influenced by the degree of polymerization; monomers, dimers and trimers have low cationic demand, whereas hexamers and long chains have a high cationic demand (Reid and Ricard, 2000). This can cause the retention devices to clog. To reduce the cationic demand, pectinase is used to degrade the polysaccharide into monomers, dimers and trimers. This helps in improving the retention of fines and filler particles in the paper sheets, resulting in a stronger and more durable paper product (Kumar *et al.,* 2012).

Fodder production

Pectinases also play a significant role in the production of animal feed or fodder. Pectin can limit the digestibility of feed for livestock, poultry and other monogastric animals. Therefore, the use of pectinases in the processing of animal feed can help to break down the pectin and increase the nutrient availability for the animals (Azzaz *et al.,* 2019). One common application of pectinases in fodder production is in the processing of sugar beet pulp. Sugar beet pulp is a by-product of sugar production from beet root and is used as a feed ingredient for livestock. However, sugar beet pulp contains high levels of pectic substances, which can limit its nutritional value (Bajpai, 2018). Pectinases can be added to the pulp during processing to break down the pectic substances and improve the digestibility of the feed. Pectinases can be used in the processing of other feed ingredients, such as soybean hulls and citrus pulp.

Biofuel production

Although lignocellulosic biofuels are a promising renewable energy resource, the recalcitrance of biomass to degradation presents a major roadblock to their production. To increase biofuel yields, one strategy is to improve the conversion efficiency of plant cell walls to bioethanol (Jordan *et al.,* 2012). The conversion process can be simplified by altering lignocellulose composition in bioenergy crop plants through genetic and molecular engineering. Pectinases can be used to break down the pectic substances in feedstocks, such as sugarcane bagasse, corn stover and other lignocellulosic materials (Somerville *et al.,* 2010). Pectinases can increase the efficiency of the hydrolysis process, allowing for higher yields of fermentable sugars that can be converted into biofuels. Pectinases are often used in

combination with other enzymes, such as cellulases and hemicelluloses, to maximize the breakdown of lignocellulosic materials. This process is commonly known as enzymatic hydrolysis and is a crucial step in the production of biofuels from lignocellulosic materials (Edwards *et al.,* 2011).

Pharmaceutical industry

Pectinases are one of the most significant industrial enzymes and some of the notable contributions in pharmaceutical sectors such as dietary or nutritive fibre preparation, essential oil extractions, drug tablet formulations, formulation for lowering blood glucose and cholesterol and cosmetic formulations (Edwards *et al.,* 2011; Sharma *et al.,* 2023). Depolymerized pectins through pectinases are used to increase stool volume and viscosity and treat diarrhoea and constipation. Enzyme treated pectic substances are utilized as a decongestant, demulcent and wound-healing aid the pectins along with sulfate can reduce clotting time and useful as an alternative to heparin (Thakur *et al.,* 2021).

Essential oils from medicinal plants are valued for their medicinal properties and are widely used in the cosmetics and perfume industries. However, the process of extracting these oils using organic solvents can damage important functional groups (Ribeiro *et al.,* 2015). To avoid this, pectinases are used in the extraction process to destroy the emulsifying properties of pectin and promote the liquefaction of cell wall components, resulting in a better yield of products (Yabukarski *et al.,* 2020). The enzymatic extraction process allows the retention of high concentrations of beneficial phytocompounds, such as polyphenols, antioxidants and vitamin E and improving their storage stability (Mwaurah *et al.,* 2019). Hydrolysed pectin beads are used as a sustained-release drug delivery system and are formulated using the ionotropic gelation method. The use of low-methoxy pectin and calcium pectinate gel beads in the formulation of the beads helps in the administration of drugs (Venkatanagaraju *et al.,* 2019). Calcium pectinate is preferred because it creates insoluble hydrophilic coatings that interact with each other in the host cell, allowing for the smooth release of the targeted medicine.

Scientists suggest that pectin has potential in preventing and treating severe diseases like diabetes and obesity. The effectiveness of pectin for this purpose depends on its viscosity, molecular weight and degree of esterification (Kashyap *et al.,* 2001). More soluble fibers are believed to enhance gut viscosity, which reduces the re-absorption of bile acids and increases the synthesis of bile acids from cholesterol, thereby improving blood cholesterol levels. Herbal formulations containing digested pectins from apple, citrus, soybean peels, etc. were tested using both in vitro and in vivo models (Kohli and Gupta, 2015).

Pectic products are commonly used in cosmetics and personal care due to their effectiveness against various skin ailments (Haile and Ayele, 2022). They are used as emulsifiers to thicken and gel face creams and their complex polysaccharides have anti-oxidative and anti-aging properties that can prevent skin damage. However, the efficacy of these products may vary depending on the source from which they are extracted or fermented using pectinase enzymes (Thakur *et al.,* 2021).

FUTURE PERSPECTIVES

Enzymes have applications in various industrial processes, such as food, feed and pharmaceuticals, and thus, justify their demand. The stability and cost of enzymes are important for their use in commercial processes. For commercialization, enzymes must be stable against harsh conditions such as elevated temperatures, organic solvents and extreme pH. Various methods are used to optimize pectinase production, but the instability of enzymes results in higher costs for their broad applicability. Research is focused on producing pectinases from selective microorganisms under specific environmental conditions. Immobilization of pectinase enzyme for reuse purposes and genetic engineering are more effective options. Future research must focus on molecular mechanisms and distinct pectinolytic enzymes with activity on the desired pectic substrates. Pectinases have the potential for significant development in various industrial processes. Protein engineering and production optimization must be considered for the successful use of microbial pectinases.

CONCLUSIONS

Microbial research has led to significant developments in finding new applications for microorganisms and their enzymes. Pectinases and pectins have been found to play important roles in various industrial processes, with promising results. However, ensuring the cost-effective production from selected microorganisms under specific environmental conditions remains a challenge. Moreover, there is a need to explore the potential of pectinase and pectic substances in the pharmaceutical industry. In order to achieve this, biotechnological approaches may be employed in developing a broad-spectrum pectinase with high catalytic efficiency with in-depth understanding of the expression mechanism at the biochemical and molecular levels are needed.

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