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BIOTECHNOLOGICAL APPLICATION OF PECTINASE

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ABSTRACT: Pectinase is a collection of enzymes that degrade pectin into simpler compounds known as galacturonic acids. Microbes and plants largely produce pectinase, and it has a wide range of biotechnological applications in food, agriculture, industry, the environment and medicine. Fruit juices, coffee, cocoa, tea, jams and jellies are all made with pectinase in the food industry. Furthermore, pectinase, a supplemental enzyme found in animal feed, increases nutrient absorption. Pectinase has been utilized in the agricultural sector for plant virus purification, oil extraction, retting and degumming processes, and cotton fiber bio-scouring. They play a critical function in wastewater treatment by facilitating pectin breakdown in the environmental sector. Pectinases are mostly used in the wine and paper industries in the industrial sector. Pectinases are probiotic enzymes that are introduced to a variety of foods in medicine. This review aims to compile all of the accessible information on pectinase enzymes' biotechnological applications in diverse industries.

INTRODUCTION: All anabolic and catabolic activities in living organisms are carried out by enzymes, which are very efficient biological catalysts. In commercial operations, enzymes are preferred over chemicals because of their strong catalytic power, a specific mode of action, environmental friendliness and low energy consumption. Microorganisms and plants both produce pectinase enzymes. Many enzyme-related research projects have been investigated worldwide for their potential use in the development of new key industrial processes. The biotechnological potential of pectinases from microbes has piqued the interest of numerous researchers across the world.

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These are wide used as biological catalysts in industrial processes [1]. As a result, pectinases are now one of the most widely employed enzymes in various industries. These enzymes are primarily responsible for the destruction of pectin, which is found as a structural polysaccharide in the middle lamella and the primary cell walls of immature plant cells [2-3]. The acquired knowledge of new microbial pectinase enzymes and methodological and technological improvements can now address the manufacture and use of these enzymes with a fresh approach, especially in recent years.

The wide range of applications and conditions in which these enzymes must function necessitates a huge number of distinct enzymes that can act in various situations. Even more intriguing would be having more robust, broad-spectrum enzymes that might be used in a wider range of applications.

Occurrence and Properties of Pectin: In non-woody tissues, pectin compounds are significant structural elements of the cell wall **Fig. 1**. They are also the only polysaccharides responsible for cell cohesion in the middle lamella [4]. Protopectin is a kind of pectin that is insoluble in water. Their synthesis begins with uridine diphosphate (UDP)-galacturonic acid and occurs mostly in the Golgi apparatus during the early stages of development, in youthful expanding cell walls [5]. The type of pectin in vegetables and fruits has a big impact on their texture. The softening of the fruits is one of the most noticeable changes during ripening. Enzymatic degradation and solubilization of protreptic chemicals are thought to cause this alteration [6].

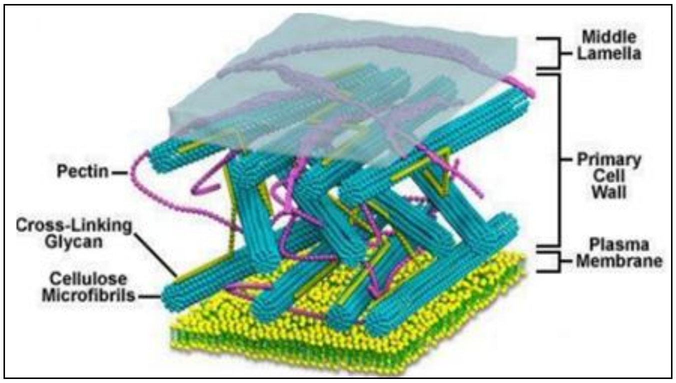

FIG. 1: PECTIN IN PLANT CELL WALL

Water, dimethyl sulphonic acid and heated glycerol are the only organic solvents that pectins are soluble in. With increasing chain length, the ease of breakdown in water reduces [7]. Water-miscible organic solvents, water-insoluble basic polymers, polyvalent cations, quaternary detergents, proteins and monovalent cations can all precipitate pectins.

The ability of pectins to produce gels with sugar and acid is one of their most unique and spectacular physical features. Calcium increases gel formation by creating strong ion connections with carboxyl groups of nearby pectin strands, known as "salt bridges."

Pectin acid is commonly used as a non-nutritive sweetener or sugar supplement in meals and beverages, particularly in diabetic diets [8]. Many of the peculiar physical features of pectic compounds are thought to be linked to the carboxyl groups of galacturonic acid residues.

Classification of Pectinase: Pectinase is a series of complex enzymes that use pectin as a substrate and are found in plants and microbes.

Pectinase enzymes are responsible for breaking down pectin, which is the main component of the middle lamella ("the cementing ingredient" of plant cell walls). This enzyme is also produced by bacteria, but mostly extracellularly. Protopectinase, the enzyme that catalyzes protopectin solubilization, was called after [9] enzymes that liberate pectin from protopectin. The liberation of pectic compounds corresponding to 1mol D-galacturonic acid/ml of the reaction mixture at 37 ^{O}C in 30 minutes is one unit of protopectinase activity.

The complete degradation of pectin requires several pectinase enzymes. These enzymes can be broadly classified into the following groups [10].

Pectin Methylesterase, PME (3.1.1.11): The first enzyme [11] of the pectin degradation pathway catalyzes the hydrolysis of the methyl ester groups of pectin into pectic acid and methanol [12].

Pectin Acetylesterase, PAE (3.1.1.6): Pectin acetylesterasecatalyzes is the hydrolysis of the acetyl ester groups of pectin molecules with the liberation of pectic acid and ethanol [13].

Polygalacturonases, PG (EC. 3.2.1.15): It is a depolymerasecatalyzing the hydrolysis of 1, 4-glycosidic linkages in linear homogalacturonan regions of pectic polymers [10]. These are of two types based on their cleavage pattern: Exo-PG and endo PG. Exo-PG cleaves through the terminal end of the pectin chain, whereas endo PG acts randomly throughout the chain [14].

Pectate Lyase, PL (4.2.2.2): It catalyzes Esther trans-elimination cleavage of α-1, 4-glycosidic linkage of pectic acid in either sequential (Exo-PL) or random manner [11].

Pectin Lyase, PNL (4.2.2.10): It catalyzes the trans-elimination cleavage of α-1, 4- glycosidic linkage of pectin molecule in a sequential manner resulting in the release of 4, 5-unsaturated oligogalacturonides [15]. In addition to the above, other enzymes catalyzing the pectin degradation are:

α–L–rhamnosidases, (EC.3.2.1.40): It hydrolyzes rhamnogalacturonan in the pectic backbone.

α–L–arabinofuranosidases (EC. 3.2.1.55): It catalyzes L–arabinose side chains by adding water molecules.

Endo–arabinase (EC. 3.2.1.99): This acts on arabinan side chains in pectin [16].

Application of Pectinase: Pectinase is primarily produced by microbes and plants and it has a wide range of biotechnological applications in the food, agricultural, industrial, environmental and medical fields.

Pectinases' Functions in the Food Industry:

Sparkling Clear Juices: Enzymes are added to sparkling clear juices to boost juice output during pressing and straining and to eliminate suspended particles, resulting in sparkling clear juices (free of haze). Treatment of the mash to achieve partial or complete liquefaction of the fruit flesh for the production of fruit pulps and nectars, to increase juice yield and improve extraction of other fruit components such as color and flavor, and treatment of the juice to reduce viscosity and enable concentration for improved clarification, filtration, and stabilization.

Mash treatment technology is detailed for producing pulps, nectars, turbid or clear juices, and their concentrates from raw materials such as apples, pears, stone fruits, berries, grapes, citrus fruits, and vegetables utilizing pectinases in combination with cellulases and hemicellulases. Clarification technology is explained, from viscosity reduction before the concentration of stable turbid juices to stabilization of clarified juices to prevent haze formation and sedimentation in the finished products, using pectinases in conjunction with amylases, proteinases and several other complementary enzymes [17].

The Antioxidant Properties of Fruit Juices Have Improved: Bioactive secondary metabolites such as polyphenol, anthocyanins, and varying levels of dietary fiber can be found in fruits and vegetables. Many cases have been researched in recent years to recognize the significance of pectinase in improving the phenolic and antioxidant content of juices and its potential health advantages. Pectinase was also employed to reduce astringency in fruit juices by solubilizing anthocyanins without causing procyanidin polyphenols to seep out [18].

Fruit Juice Clarification: When pectinase is applied to fruit pulp, pectin is destroyed, and the viscosity of the juice is lowered, making it easier to handle. Pectinase is involved in the maceration, solubilization and clarifying of fruit pulps. Treatment with pectinolytic enzymes decreases viscosity and turbidity quickly by aggregating hazy particles and settling them out. Filtration or centrifugation can be used to separate the juice from the particles. The final product is a sparkling, transparent juice [19].

Careful experimentation with the purified enzymes has led to the conclusion that the clarification process is a combined effect of pectin methylesterase and polygalacturonase [20] or pectin lyase alone in the case of apple juice, which contains highly esterified pectin (>80%) [21]. in grape juice, which contains pectin with a lower degree of esterification (44%–65%), pectin lyase alone does not perform as well [22] Recently used S. cerevisiae, yeast, producing pectin degrading enzymes for the clarification of fruit juices [23].

Coffee, Cocoa, and Tea Fermentation: Pectinases are enzymes that are used in a variety of fermentation processes. They help to speed up the fermentation process. Pectinase enzymes of fungal origin break down pectin found in tea leaf cell walls during fermentation, but too many enzymes can harm tea leaves, so a specific concentration must be maintained. They also work as antifoaming agents in instant tea powders by degrading pectin [24].

Fermentation pectinases remove the mucilage layer from coffee beans during the roasting process. Enzyme pectinase preparations are sprayed onto cocoa beans to carry out the fermentation. The filtrate of inoculated fermentations is a less expensive alternative that accomplishes the same goal. On the other hand, enzymatic pectinases are more effective since the enzymes speed up the fermentation process [25-26]. To generate the chocolate flavor, cocoa must be fermented. Many different microorganisms, including pectolytic microbes, play a part in cocoa fermentation. By releasing pectinases, these microorganisms aid in the decomposition of the cocoa pulp, resulting in the highest grade cocoa beans with premium chocolate flavor [27-28].

Preparation of Jams and Jellies: By demethylation, pectinesterase transforms high methoxylatedpectins to low methoxylatedpectins. Pectin esterase possesses a calcium-dependent gelation characteristic, which reduces sugar consumption by forming a gel. Jellies, jams, compotes, sauces and soups are all made with this enzyme [29].

Pectinase in Agricultural Sector:

Purification of Plant Viruses: Plant viruses are isolated with the help of pectinases. Plant viruses are released from the phloem tissue by alkaline pectinases combined with cellulases, resulting in a pure preparation of plant viruses.

Oil Extraction: Oil extraction from plants uses pectinases, although olive oil extraction is the most prevalent. Cellulases and hemicellulases are used in commercial olive oil extraction procedures. These enzymes are given to olives after grinding to facilitate oil extraction. Pectins prevent emulsification by interfering with the gathering of oil droplets from peel extracts, which aids oil extraction in the case of lemons [30]. Sunflower, coconut, lemon, palm, or canola vegetable oils can also be extracted using organic solvents such as hexane and pectinases, particularly of an alkaline nature, which aid in the extraction of oils in an aqueous process. Oil yield and stability are improved as a result of enzyme treatment. Olivex is a commercial enzyme preparation made from the fungus Aspergillus aculeatus. It exhibits pectinolytic, hemicellulases and cellulolytic activity, all of which are desirable for high oil output and storage stability. Olivex treatment results in a high amount of polyphenols and vitamin E content and an improvement in the organoleptic quality of the oil, which must be determined before the influence on oil consumption, can be determined [31]. Olive oil is now being made with a plant cell wall-degrading enzyme preparation. The enzyme is added during the grinding of olives, allowing for easier oil removal during subsequent separation steps.

Plant Fiber Retting and Degumming: In the degumming of fiber crops, pectinases play a critical role. The fiber obtained from these crops contains gum; ramie fiber is a great natural textile, but it contains 20-35 percent ramie gum, which is primarily composed of pectin and hemicellulose, and so must be removed before further processing. Degumming can be accomplished with either a chemical or an enzymatic process. To remove the gum from decorticated fibers during chemical treatment, 12–20 percent NaOH in solution is utilized [32]. However, chemical treatment is ineffective, and this sort of degumming is hazardous, polluting, and non-biodegradable. Enzymatic treatment is more effective; pectinases and xylanase can be employed to degum in an environmentally benign and biodegradable manner [13]. The use of enzymes also minimizes the number of chemicals and energy used. Pectate lyase from actinomycetes is the most effective pectinase for separating bast fiber by eliminating gums. Retting is a fermentation process in which pectin is destroyed and eukaryotic and prokaryotic bacteria release pure quality fiber. Clostridium and Bacillus are the most typically employed bacteria in retting, whereas Aspergillus and Penicillium are the most commonly utilized fungi. Water retting of flax requires bacterial pectinases, which causes fiber separation [33].

Bio Scouring of Cotton Fibers: Natural cotton fibers include non-cellulosic contaminants that can be removed using particular enzymes in a process known as bio-scouring. Non-cellulosic impurities are removed from cotton using commercial preparations produced from pectinases, lipases, amylases, cellulases, and hemicellulases effectively and safely. **Table 1** demonstrates the range of applications for those enzymes that are both biodegradable and remove non-cellulosic contaminants. Because enzymes are biodegradable, this procedure is also environmentally friendly. They are the most effective alternative to the traditional way of using caustic soda without causing cellulose degradation [2]. The traditional scouring technique uses harsh chemicals and is gradually being replaced by an environmentally friendly approach that uses enzymes. Bio-scouring is an environmentally acceptable method for removing non-cellulosic contaminants from fiber using enzymes [34]. It makes the fibre surface more hydrophilic [35]. Bio-scouring also avoids high energy consumption and severe pollution problems that are associated with conventional alkaline scouring [36] Pectinases prevent fiber damage [37].

TABLE 1: ENZYMES IN THE PROCESSING OF TEXTILE AND AREA OF APPLICATION

Enzyme	Textile Substrate	Area of Application
Amylase	Starch	Desizing
Cellulases	Cellulose	Bio-polishing and Denim Washing
Pectinase	Pectins	Bio-scouring
Catalase	Peroxides	Bleaching
Lipase	Oils and fats	PET hydrophilicity improvement and Detergent additive
Laccases	Dyes and intermediates	Effluent treatment
Proteases	Proteins	Wool Scouring and Degumming of silk

Animal Feed: The use of pectinases in the manufacturing of bovine feed reduces feed viscosity and promotes ruminant nutrient absorption, liberates nutrients by enzymatic action, and reduces stool output [2, 34] Improvements in animal performance owing to enzyme additions can be attributed mostly to improved ruminal fiber digestion, which leads to increased digestible energy intake [38].

The use of enzymes in feed has increased animal weight. Treatment of feed with enzymes shortly before feeding allows for more management options. This type of enzyme treatment may improve feed digestibility through a variety of methods, including direct hydrolysis, palatability improvements, and changes in gut viscosity [39].

Pectinases in the Industrial Sector:

Pectinases in the Wine Industry: Wine is made from grapes that have been fermented. Numerous enzymes are involved in the winemaking process **table 2**, including hemicellulases, glucanases, and glycosidases.

Of these, pectinases play a significant role in the winemaking business. Because of PME [40], they raise the level of methanol in wine. Methanol levels should be monitored since excessive amounts might be hazardous. As a result, pectinases with poor pectin methylesterase activity are seen in commercial combinations.

Pectinases help to extract wine by boosting juice yield, speeding up filtering, and enhancing the wine's flavor and color. Pectinases improve the wine's fragrance and flavor. Grapes feature a variety of aromatic components that exist in free form and give off a scent when linked together or in higher concentrations. Some non-odorous glycoside taste precursors begin to accumulate in grape skin throughout the ripening process. When pectinases are added to the wine and must extraction and fermentation processes, they increase the number of aromatic precursors in the grapes, making them more susceptible to hydrolysis when attacked by -glucosidases, which are produced by microbial fermentation, increasing the aroma of the wine [41].

The extraction of pigments improves the color yield when pectic enzymes are used. Many suspended particles and certain undesirable bacteria are settled out of the juice when it is treated with pectic enzymes before or during fermentation.

As a result, the wine is clearer, and the yeast sediment is harder. Pectic enzymes are added to fermented wine to boost the filtration rate and clarity.

Table 2 winemaking enzymes are obtained from grapes and wine-associated microorganisms [42]. Grape pectinases are inactive under the pH and SO_2 conditions associated with winemaking.

Fungal pectinases are resistant to these winemaking conditions. The pectinase family is responsible for the majority of the current activities employed in winemaking preparations.

Pectin lyase (PL), pectin methyl-esterase (PME), and polygalacturonase are some of them (PG). Depolymerizing activity, also known as pectin lyase, involves cutting the pectin chain between two galacturonic methylated acids, whereas the PG prefers a non-methylated substrate. The methylic group from galacturonic esterified acids is released by pectin methyl-esterase activity, which does not depolymerize the pectin chain [43].

Pectinase was used to improve the stability, flavor, and structure of red wines. Yield, anthocyanin level, total phenolics, tannins, clarity and color intensity were all shown to be higher in wines made with pectinase [10].

TABLE 2: ENZYMES DERIVED FROM GRAPES AND WINE-ASSOCIATED MICROBES INVOLVED IN WINEMAKING

Enzymes	Remarks
Grapes (Vitisvinifera) Glycosidases	Hydrolyze sugar conjugates of tertiary alcohols; inhibited by glucose; optimum pH 5-6
Protopectinases	Produce water-soluble, highly polymerized pectin substances from protopectins
Pectin methylesterases	Split methyl ester groups of polygalacturonic acids, release methanol, convert pectin to pectate; thermo-stable; opt. pH 7-8
Polygalacturonases	Hydrolyse α-D-1,4-glycosidic bonds adjacent to free carboxyl groups in low methylated pectins and pectate; optimum pH 4-5
Pectin lyases	Depolymerise highly esterified pectins
Proteases	Hydrolyses peptide bonds between amino acid residues of proteins; inhibited by ethanol; thermostable; optimum pH 2
Peroxidases	Oxidation metabolism of phenolic compounds during grape maturation; activity limited by peroxide deficiency and SO2 must
Fungi (Botrytis cinerea) Glycosidases	Degrades all aromatic potential of fungal infected grapes
Laccases	Broad specificity to phenolic compounds, cause oxidation and Browning
Pectinases	Saponifying and depolymerising enzymes, cause degradation of plant cell walls and grape rotting
Cellulases	Multi-component complexes: endo-, exoglucanses, and cellobiases; synergistic working, degrade plant cell walls
Phospholipases	Degrades phospholipids in cell membranes
Esterases	Involved in ester formation
Proteases	Aspartic proteases occur early in fungal infection, determine the rate and extent of rotting caused by pectinases; soluble; thermostable
Yeast (Saccharomyces cerevisiae) β-Glucosidases	Some yeast produce β-glucosidases which are not repressed by Glucose
β-Glucanases	Extracellular, cell wall-bound, and intracellular, glucanases; accelerate the autolysis process and release mannoproteins
Proteases	Acidic endoprotease A accelerates the autolysis process.
Pectinases	Some yeast degrade pectic substances to a limited extent; inhibited by glucose levels < 2%
Bacterial (Lactic acid bacteria) Malolactic enzymes	Convert malic acid to lactic acid
Esterases	Involved in ester formation
Lipolytic enzymes	Degrades lipids

Paper and Pulp Industry: Chemicals were widely used in the pulp and paper business to make paper, but enzymatic technologies and procedures made this process easier to achieve better and more efficient results. Different raw materials are utilized in traditional paper businesses. During the continuous filtration process, a diluted suspension including fiber fragments and inorganic particles like clay or $CaCO_3$ is generated, which is then molded into sheets. Large pores in filter textiles allow fines and filler particles to flow through readily. These particles are kept in paper sheets in modern paper-making enterprises to allow for rapid water drainage. The most popular retention aids are cationic polymers [44]. Alkaline peroxide bleaching of pulps solubilizes polysaccharides, interfering with paper production [45]. However, using pectinases in paper production minimizes the requirement of cationic polymers and pulp bleaching. *Streptomyces* sp. alkaline pectinase was used to improve the bleaching of the eucalyptus pulp. Bio bleaching enzymes generated by the same fungus species are pectinase and xylanase.

Pectinase in the Environmental Sector:

Pretreatment of Pectic Wastewater: Multiple methods are used to treat wastewater from citrus processing companies that contain pectic compounds, including physical dewatering, chemical coagulation, direct activated sludge treatment, and chemical hydrolysis, which results in the generation of methane. These have various drawbacks, including expensive treatment costs and prolonged treatment durations, and environmental damage from chemical use. As a result, using pectinases from bacteria, which selectively remove pectic compounds from wastewater, is an alternative, cost-effective and ecologically acceptable way. The use of alkaline pectinase and alkalophilic pectinolytic microorganisms to pre-treat pectic wastewater from vegetable food processing companies makes it easier to remove pertinacious material and makes it acceptable for decomposition by activated sludge treatment. An extracellular endopectatelyase from an alkalophilic soil isolate, *Bacillus* sp. GIR 621 was used effectively to remove pectic substances from industrial wastewater [46].

Pectinase in the Medical Sector:

Prebiotics / Functional Foods: According to the definition of a prebiotic, it is "a selectively fermented material that allows specific alterations in the makeup and/or activity of the gut microbiota to boost the host immune system [47]. In recent years, pectinase has been employed in several ways, including a prebiotic component and a functional food [48]. Pectin and pectin-derived oligosaccharides (PDO) are emerging as promising possibilities for next-generation prebiotics. Intestinal bacteria have been found to metabolize demethylated pectin more quickly to create short-chain fatty acids (SCFA), such as the health-promoting acetate, propionate, and butyrate [49; 47]. By competing with natural galectins, oral feeding with galactose-containing citrus pectin (heat-treated) suppresses spontaneous prostate carcinoma spread and promotes apoptosis in cancer cells [50].

CONCLUSION: Biotechnological solutions for environmental sustainability are current solutions that aid in the nation's development and are a boon to current and future generations' human wellbeing. Biotechnology's use of industrial processes for enzyme synthesis is no longer an academic or potentially advantageous future goal. Pectinases are new enzymes that are involved in pectin degradation. Because of their wide range of applications, pectinases are particularly essential in various industries. Pectinases account for 25% of all commercial enzymes sold each year globally. Plants, bacteria, and fungi, for example, can easily provide these enzymes. Pectinases are widely used in the food and agriculture industries. Aside from these applications, they have many industrial and medicinal applications. Pectinases have received a lot of attention in the industry, where they've successfully replaced many harmful substances in the environment. Pectinases are one of the most rapidly expanding enzymes in the biotechnological industry, with consistent demand. They are at the top of the list of industrial enzymes that are commercially available.

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