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Pectinase: a comprehensive review of sources, structural property, catalytic mechanism and production with microorganism from agrowaste.

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ABSTRACT :

The complex polysaccharide pectin, which is present in plant cell walls, is broken down by the enzyme pectin lyase. Numerous microbes, such as bacteria and fungi, synthesize this enzyme, which they then secrete into the environment. The production of pectin lyase can be affected by variables such as pH, temperature and nutrient availability. To increase pectin lyase production in microorganisms, researchers have investigated genetic engineering strategies. Pectin lyase is used in various industrial processes, such as the clarification of fruit juices and the formulation of pectin-containing goods. By understanding the variables affecting pectin lyase synthesis and optimizing its production conditions, processes in various fields can be made more efficient and sustainable. And in this review paper we discuss its structure, shape, enzyme containing it, its source, its utility, production from agro waste and purification.

Keywords: Pectinase , Pectin , Agrowaste , Enzyme

Introduction :

Plant pathogens assault target cells by releasing a variety of enzymes that break down the cell wall, allowing the pathogen to enter and spread throughout the host tissue. These enzymes include pectin lyase, cellulases, and proteases, among others. According to the specificity of the substrate and the mechanism of action, pectin lyase are a group of enzymes that are divided into a number of groups and subclasses, such as methyl esterase, hydrolases, and lyases. One of the first tasks carried out by the majority of fungal pathogens during plant infections is the degradation of pectic polymers, which make up the majority of all dicots and some monocots primary cell walls. **[4]**

Pectin lyases are particular importance among pectin lyase since they degrade pectin polymers immediately through b-elimination, leading to the generation of 4,5-unsaturated oligogalacturonides, as opposed to other pectin lyase that function sequentially to entirely degrade pectin molecules. Pectin lyase plays a role in microbial phytopathogenesis in addition to having biotechnological potential in the fruit juice industry because it breaks down pectin without affecting the ester group, which is responsible for the juice's pervasive, and it avoids toxic methanol formation **[3]**. This enzyme may also be used in the textile industry to degum and retie natural fibers like flax, ramie, hemp, and jute **[4]**. The biochemical characterization, production, and applications of pectin lyase have been investigated. **[5–11]**, but as of yet, there hasn't been a review article in the literature specifically on pectin lyases. In this essay, we've made an effort to close that gap. Since pectin serves as the substrate for pectin lyase, it is crucial to briefly go over the key aspects of its structure and makeup.**[2]**

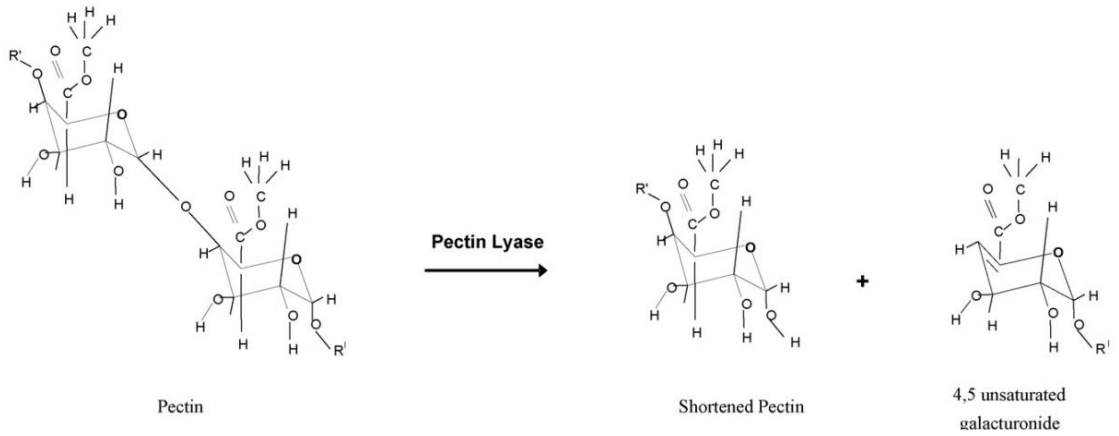

Fig.1. Mechanism of action of pectin lyase. [1].

Enzymes related to pectin lyases :

Pectin breakdown is carried out by a class of pectinolytic enzymes known as pectin lyase. These enzymes have undergone numerous reviews. These enzymes can be categorised succinctly into the groups listed below based on their preferred substrate and method of action. Due to the enormous variety in the structure of pectin, they can be divided into enzymes operating on the "hairy region," which is made up of rhamnogalacturonan(a family of pectic polysaccharides that contain a backbone of the repeating disaccharide) and side chains, and enzymes acting on the "smooth regions," formed of homogalacturonan. Rhamnogalacturonan hydrolase (RG hydrolase), rhamnogalacturonan lyase, rhamnogalacturonan rhamnohydrolase, and rhamnogalacturonan galactohydrolase (RG galactouronohydrolase) are the groupings of enzymes that are involved in the degradation of the hairy area of pectin **[19]**. The structures and functions of these enzymes need to be thoroughly explored because they haven't been studied very often. However, other auxiliary enzymes, including as aarabinofuranosidase, endoarabinase, b-galactosidase, endogalactanase, and feruloyl and p-coumaroyl esterases, are also involved in the degradation of the side chains of pectin **[27]**.

Table 1: Types of Pectinase and their mode of action

Type of pectinase	Substrate	Mode of action	Product
1.Esterases			
(a)PME	Pectin	Hydrolysis	Pectic acid + methanol
(b) PAE	Pectin	Hydrolysis	Pectic acid + ethanol
2.Depolymerases			
(a) Hydrolases			
(i) Endo PG	Pectic acid	Hydrolysis	Oligogalacturonates
(ii) Exo PG	Pectic acid	Hydrolysis	Monogalacturonates
(b) Lyase			
(i) Endo PL	Pectic acid	Transelimination	Unsaturatedoligogalactouronates
(ii) Exo PL	Pectic acid	Transelimination	Unsaturated digalactouronates
(iii)EndoPNL	Pectic	Transelimination	Unsaturated methyloligogalactouronates

*PME, pectin methyl esterase; PAE, pectin acetyl esterase; PG, polygalacturonase; PL, pectate lyase; PNL, pectin lyase.**[4,7,43]**

Sources of pectin lyase :

Despite reports of significant production in the absence of its natural inducer, PNL is a highly inducible enzyme like all pectin lyase [17]. Pectin lyases have primarily been researched in microbes, while there are sporadic reports of their occurrence in plants and mammals as well [6]. Although there have been a few studies on bacterial and yeast PNLs, the majority of PNLs are produced by the fungi Aspergillus, Penicillium, and Fusarium. The type of medium (natural or synthetic) employed to produce PNL by Penicillium italicum CECT 2294 has an impact on its production [13]. Phaseolus vulgaris isolate Colletotrichum lindemuthianum was discovered to produce two types of PNL when cultivated in vitro with sodium polypectate or in a media containing Phaseolus vulgaris cell walls [11]. In a medium containing sugarcane juice, methylxanthines, yeast extract, sucrose, and tea extract, Penicillium grieseoroseum can create PNL [19,17]. Pythium splendens was found to produce a pectin lyase in infected cucumber fruit [8]. In a liquid culture media, Rhizoctonia solani AG 8 generates pectin lyase [9]. Additionally, it has been noted that Penicillum and Lasidioplodia species produce pectin lyases in liquid culture medium supplemented by pectin [10]. In a liquid growth medium that contained 12 g of sucrose or 15 ml of glycerol as supplements, Pseudomonas fluorecens E51 naturally formed PNL [14]. A small number of researchers have also shown that DNA-damaging chemicals like mitomycin C (MC), nalidixic acid, or bleomycin added to the media or UV radiation exposure of cells both stimulate PNL synthesis [4].
Aspergillus sojae, Erwinia aroideae, Aspergillus niger ,Aspergillus japonicus,Alternariamali ,Penicillium paxilli, Aspergillus oryzae ,Colletotrichum lindemuthianum,Pseudomonas marginalis,Rhizoctonia solani, Fusarium oxysporum, Bacillus sp.PN, Cystofilo basidiumcapitatum, Penibacillus amylolyticus, Penicillium canescens,Penicillium expansum, Penicillium italicum, Penicillium viridicatum, Pythium splendens, Pseudomonas fluorescens, Rizopusoryzae, Aspergillusflavus, Aspergillusficuum etc.

Table 2: Different microorganisms and its sources: Extracellular pectinase production

Name *of* Microorganisms	Source	Morphology	Reference
Bacillus pseudofirmus	Hotsprings	Gram positive, aerobic endospore forming bacteria	**[7]**
Paenibacillus sp.	Soil samples	Gram positive, rod shaped, endospore forming bacteria	**[7]**
Bacillus licheniformis	Hotsprings	Gram positive, rod	**[7]**

		shaped	
Bacillus clausii	Soda lake	Gram positive, rod shaped, spore forming bacteria	**[7]**
Bacillus sonorensis	Spoiled fruits and vegetables	Gram positive, rod shaped, spore forming bacteria	**[7]**
Geomyces species	Antartica	Filamentous with branched conidiophores	**[7]**
Aspergillus tubingensis	Soil of vineyards	Filamentous fungi with black spores	**[7]**
Aspergillus awamori	Agricultural wastes	Filamentous fungi with conidiophores	**[7]**
Aspergillus flavus	Soil and decomposing orange peels	Filamentous with conidia	**[7]**
Aspergillus niveus	Mangifera Indica	Globose, ellipsoidal or cylindrical	**[7]**
Kluveromyces marxianus	Grape juice	Diploid or haploid forms.	**[7]**
Saccharomyces cerevisiae	Grape skin	Spherical to ellipsoid shape with Pseudohyphae	**[7]**
Wickerhanomyces anomalus	Citrus fruit peels	Form ascospores	**[7]**

4.Structural aspects of pectin lyase :

Despite only having 17% sequence identity after pairwise structure-based alignment, pectin lyase A (PNLA) from two strains of Aspergillus niger, N400 and 4M-147, folds into a parallel b sheet and shares many structural characteristics with pectate lyases, according to the crystal structures of PNLA [20]. Amino acid stacks and the asparagine ladder are two examples of these structural similarities. These two PNLs have aromatic residue-dominated substrate-binding clefts that are surrounded by negative electrostatic potential. The conformation of the loop created by residues 182-187 makes up the majority of the variation between these two PNLA structures. These observed variations result from the various crystallization pH levels. Pectin lyase B (PNLB) from Aspergillus niger has also had its three-dimensional structure determined using crystallographic techniques [9] with a resolution of 1.7 A.

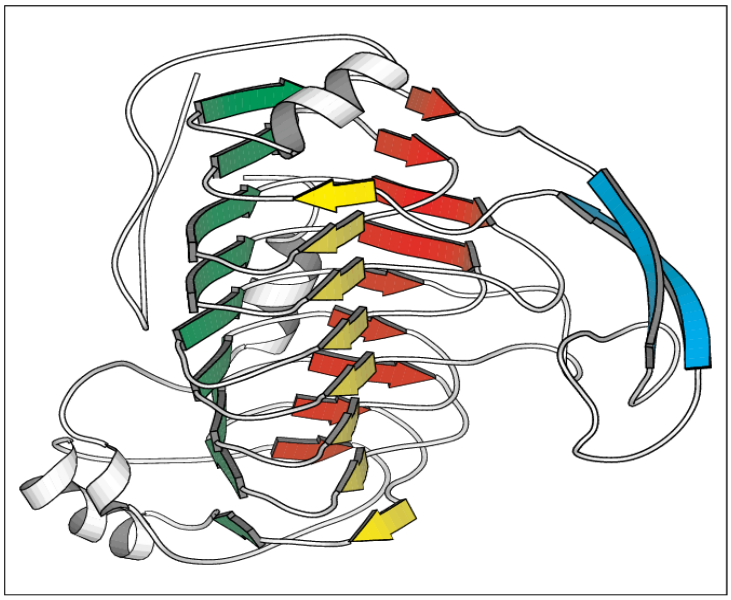

Fig 2. Structure of pectin lyase [3]

5. Production of pectin lyase from various agro-industrial waste

During the raw agriculture material processing for food, a bulk quantity of agro-wastes generated, Thus, agro residues of coffee, orange, rice, and sugarcane provide a suitable platform for bio-production of different enzymes using the fermentation process, thereby making valuable of these waste products**[6].** Orange bagasse contains large amounts of soluble carbohydrates, particularly fructose, glucose, sucrose, and pectin, as well as insoluble cellulose, and has been used as a fermentation product including enzymes **[2].** These enzymes are used for increasing juice extraction by decreasing the viscosity of concentrates in cellulose fiber preparation, coffee/tea fermentation, oligogalacturonides production and for the clarification of juices and wines **[16,17]**

Polygalacturonase (PG) is an important member of this group produced by many bacteria, fungi, and some specific plant cells **[4]**. Most of the pectinolytic enzymes are produce by fungi, however different species of genus Bacillus are also capable of producing these enzymes. Bacillus strains adapt changes in the growing conditions more easily and these factors contribute to better production of enzymes. Several Bacillus species have been reported to produce PG enzymes such as *B. subtilis*, and *B. licheniformis* **[4]**. These bacteria are responsible for approximately 50% of the total enzymes production **[7].**

There will be a tremendous gain to the industry if some byproduct from industries is used as substrate for microbial production of pectinase. Such an approach would be highly economical and may lead to cost reduction of the end product. Such a possibility may also prove useful in overcoming the present economic difficulties faced by the coffee processing industry. One of such examples is waste from the coffee industry. The presence of around 6.5% pectin in coffee pulp on a dry basis, make this waste, a good substrate for pectinase production **[4].** PGs are also important in some other fungi and bacteria virulence like Aspergillus flavus, Claviceps purpurea, Agrobacterium tumefaciens and Ralstonia solanacearum. Fungi like Aspergillus Niger, Aspergillus oryzae, Penicillium expansum, which are Generally Regarded As Safe (GRAS) by United States Food and Drugs Administration (USFDA) are employed in the food industry [19]. Microorganisms have noteworthy collaboration in food (dairy & meat) and alcoholic beverages industries **[1].** The usage of bio-produced food and additives makes them more appropriate rather than the synthetically produced ones **[11].**

The process known as "solid-state fermentation" (SSF) uses a solid matrix and takes place when there isn't much fluid present in the area between the substrate's particles. Water is present in the solid substrate in this system, whose ability to retain liquid varies depending on the type of material. In contrast, the nutrients and bacteria are both submerged in water during submerged fermentation (SmF) **[2].**

Many naturally synthesized cellular components of plants, animals, and microorganisms help meet some human nutritive and functional needs **[13]**. A number of these useful compounds occur as biopolymers, mainly polysaccharides and a few proteins (collectively called hydrocolloids, being water-loving polymers). Pectin is a native polysaccharide in the cell wall and middle lamellae of many land-growing plants, especially those of fruits and vegetables. Historically, the compound was dubbed 'pectic acid' from the Greek word πηκτικός pēktikós, meaning coagulated material, by the French scientist Henri Braconnot, who first isolated pectin from vegetables in 1825 **[6]**. However, one of the questions raised regarding the conventional pectin production process, particularly the extraction step, is whether the valorization of fruit and vegetable processing by-products is worth energy and economic demands that are currently associated with the practice **[21].**

The benefits and drawbacks of employing one or the other fermentative technique have been extensively discussed [3]. In comparison to SmF, it has been proposed that SSF enhances the amount of enzyme obtained **[4, 5]**. According to several studies, SmF's catabolite suppression makes enzyme production more sensitive **[6, 7]**. The type of fermentative procedure employed for the creation of microbial extracellular protein molecules also affects their intrinsic features, such as temperature and pH maxima for activity, thermostability, stability in various pH ranges, and substrate affinity **[8, 9].** Additionally, Aguilar et al. **[4]** found that SSF and SmF may express proteins differently.

Waste material from agroindustrial processing may be used as the substrate for microbial growth in SSF or SmF. The organic matter in this material is used as a source both of energy for growth and of carbon and nutrient for synthesis of cell biomass and other products of microbial metabolism, so that the waste is upgraded and valuable products may be synthesized **[10, 11].**

Analytical grade materials were utilized throughout. The agricultural waste materials, including municipal paper waste, wheat straw waste, apple pomace waste, lemon peel waste, and orange peel waste, were gathered from a nearby fruit market and regularly discarded local newspapers, respectively **[5].**

Table 3: Comparison of pectinase yield by different types of reactors used for production

Type of reactor & Process	Substrate	Yield	References
Stirred tank bioreactors with dual Rushton turbine impeller & Submerged fermentation	Lemon peel	Exo polygalacturonase:462Ug^{-1}	**[33]**
Stirred tank bioreactor & Submerged fermentation (Both pectin lyase and pectate lyase)	Lemon peel powder and galactose	Pectin lyase:23,300U/L& Pectate lyase:22,400U/L	**[34]**

Pilot scale packed bed reacto & Solid state fermentation	Citrus waste and sugarcane bagasse	33 to 41Ug^{-1}	**[25]**
Pilot scale packed bed reactor & Solid state fermentation	Wheat bran and sugarcane bagasse	22Ug^{-1}	**[36]**
Pilot scale packed bed reactor & Solid state fermentation	Wheat bran and sugarcane bagasse	17 to 20 Ug^{-1}	**[5]**
Rotating bed reactor & Solid state fermentation (Both amyloglucosidase and exopolygalacturonase)	Defatted rice bran	Exopolygalacturonase: 84Ug^{-1} & Amyloglucosidase: 886.25Ug^{-1}	**[17]**
Trickle bed reactor & Solid state fermentation	Wheat straw	Fungal Phytase: 41.25 $Ugds^{-1}$	**[38]**
Column tray type bioreactor	Lemon peel	Pectinase:	**[9]**

6.Purification of pectin lyase enzyme :

Size exclusion chromatography, hydrophobic interaction, and ammonium sulfate precipitation were used to purify the pectin lyase enzyme from Bacillus pumilus BK2.With a recovery of 19, the purifying procedure led to a seventeen-fold increase in specific activity.SDS-PAGE validated the homogeneity of the enzyme's purification, and it had a molecular mass between 37.3 and 4.8 kDa. Pectin lyase from Bacillus pumilus BK2 was used for the enzymatic pectin removal, and it shown the capacity to remove up to 80% of the pectin from cotton's outer layer[14].

Ammonium Sulfate Precipitation:

In order to selectively precipitate proteins, ammonium sulfate is added to the crude extract in this phase. Differential precipitation can be used to some extent purify pectin lyase.[40]

Ion Exchange Chromatography:

Proteins are frequently separated using ion exchange chromatography according to how charged they are. Ion exchange resins can be used to bind and elute pectin lyase selectively[14].

Gel Filtration Chromatography:

Proteins are separated via gel filtration chromatography, with bigger proteins eluting first. Based on its size, pectin lyase can be further purified using gel filtration chromatography[14].

Affinity Chromatography:

In some cases, affinity chromatography using pectin or other ligands can be employed to achieve high specificity and purity[14].

Table 4: Different purification methods which are used during pectinase production processes in industries [6,21,37,21].

Organism & Type of enzyme	Purification method	Purification fold	Specific activity (U/mg)	Yield (%)	Reference

Aspergillus niger & Pectinase	Ammonium sulphate precipitation	1.36	23.6	15.11	**[34]**
	Dialysis	2.87	46.9	16.33	
	Gel filtration chromatography	5.59	97.2	12.96	
Neurospora crassa & Polygalacturonase	Ethanol precipitation	1.35	7.17	42.72	**[24]**
	Gel filtration Chromatography	56.14	298.65	28	
Bacillus sp.ZJ1407 & Acidic pectinase	Ammonium sulphate precipitation	5.29	4.87	67.2	**[24]**
	DEAE-cellulose	83.32	76.66	8.17	
	Sephadex G-100	120.07	110.47	5.00	
Aspergillusfumigatus & Pectinase	Ammonium sulphate precipitation	1.22	10.66	68.09	**[38]**
	Ion exchange chromatography	1.74	15.19	27.36	
	Sephacryl-200 gel filtration	4.45	38.88	26.16	
Schizophyllum commune & Pectinase	Ammonium sulphate precipitation	1.22	141	11.93	**[15]**
	Dialysis	1.4	162	8.81	
	Sephadex G-100 column	3.08	355	4.16	
Paenibacillus sp & Pectinase	Acetone precipitation	6.25	12.42	2.24	**[37]**
	Sephadex G-75	97.65	187.66	3.73	
Aspergillus niger & Pectinase	Acetone precipitation	1.2	8.33	19	**[6]**
	Sephadex G-75	8.5	60	16	

7.Application of pectin lyase :

1. **Acidic pectin lyase:Acidic pectic enzymes used in the fruit juice industries and wine making often come from fungal sources, especially from Aspergillus niger.**

The juices produced by these industries commercially include:

A. Sparkling clear juices, (apple, pear and grape juices,
B. Juices with clouds (citrus juices, prune juices, tomato juice and nectars),
C. Unicellular products to preserve the integrity of the plant cells by selectively hydrolyzing the polysaccharides of the middle lamella [7].

2. **2.Sparkling clear juices**

Enzymes are added to increase juice yield during pressing of juice and to remove suspended matter to give sparkling clear juices.

- Cloudy juices

Pectin enzymes containing high levels of polygalacturonase activity are added to fruit juices to stabilize the cloud of citrus juices and nectars.

- Unicellular products

Formed by transformation of organized tissues into a suspension of intact cells, resulting in products used as base material for pulpy juices, baby foods, puddings and yogurt. Enzymes used for this purpose are referred to as "macerases"**[7].**

3. **3.Alkaline pectin lyase**

Alkaline pectin lyase are mainly used in the degumming and retting of fiber crops and pretreatment of pectic wastewater from fruit juice industries.These enzymes come mostly from bacterial sources. In the industrial sector, alkaline pectin lyase, mainly from *Bacillus spp*. are applied**[7]**.

4. **4. Retting and degumming of fiber crops:**

Pectinolytic enzymes are involved in the retting anddegumming of jute, flax, hemp, ramie and coir from coconut husks. Retting is a fermentation process in which certain bacteria (*Clostridium, Bacillus*) and certain fungi (*Aspergillus, Penicillium*) decompose the pectin of the bark and release fiber**[16].**

5. **5.Treatment of pectic wastewater:**

The wastewater from the citrus-processing industry contains pectinaceous materials barely decomposed by microbes during the activated-sludge treatment. A soft-rot pathogen, Erwinia carotovora secrets endo-pectate lyase in the pretreatment of pectinaceous wastewater **[16].**

6. **6. Production of Japanese paper**:

Alkaline pectinase produced by *Bacillus sp*. and Erwinia carotovora has been used for retting of bast. These retted bastes have been used for the

preparation of Japanese paper. The strength of the pulp from bacterial retting is as high as that obtained by the conventional soda-ash cooking method. The paper sheets prepared from this pulp are very uniform and soft to touch **[16].**

7. 7. Oil extraction:

Pectinase are used to extract vegetable oil in an aqueousprocess by liquefying the structural cell wall components of the oil-containing crop. The enzymes are added during grinding of the seeds the oil is released easily in the separation techniques. This increases the yield. Olivex, an enzyme preparation derived from A. aculeatus, contains pectinolytic activity and give good oil extraction and better stability when stored. The oil also shows increased content of polyphenols and vitamin E, which stabilizes the oil**[16]**.

8. 8.Coffee and tea fermentation:

Pectin lyase play an important role in coffee and tea fermentation. Fermentation of coffee using pectinolytic microorganisms is done to remove the mucilage coat from the coffee beans**[16].**

8.Future perspectives on the research and industrial use of pectinase:

Globally, there is a growing need for pectinases for a variety of applications. To meet this demand, numerous strain improvement techniques (such as metabolic, genetic, or protein engineering) are available. Naturally occurring wild type strains of organisms make up the majority of the organisms used in industry. In the future, this demand for pectinase may be further satisfied by genetically modified organisms (GMOs), altered strains, and laboratory chosen mutants that are obtained by strain enhancement methods and have improved capabilities. In order to produce durable pectinolytic mutants, these organisms undergo a variety of physical treatments (such as X-rays, UV radiation, and gamma rays) or chemical treatments (such as Ethidium bromide, colchicine, and hydrogen peroxide)**[24]**.The choice of compound mutation approaches may have synergistic effects across the genome, adding to the complexity of the gene's overall function **[26]**. The strategies for strain enhancement need to be examined across many generations because they can lead to more favorable business chances. In the future, more emphasis must be placed on the studies confirming the safety of GMO laws through study and application evidences. The development of trustworthy techniques, which are essential for enhancing the stability of the enzyme, must also be a focus of research. It is possible to produce these enzymes with longer storage times at low cost as their stability increases. In these situations, immobilizing enzymes over affordable matrices might be a superior long-term option since it will increase productivity by allowing for long-term storage stability and effective pectinase utilization. Extremophiles may also attract more attention since they give enzymes a wider range of pH and temperature tolerance as well as faster response rates**[12]**. Using high cell density perfusion cultures for pectinase production in upstream processing may result in increased production of pectinase with high activity. Online digital holography, impedence flow cytometry, and dielectric spectroscopy might all be utilized to track strains' cell development both online and offline **[35].**

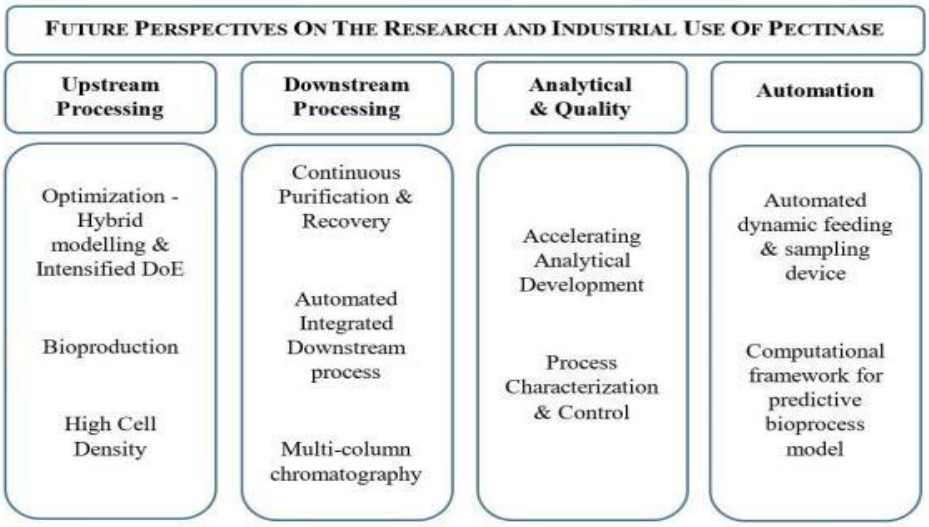

Figure 4: Future perspectives on the research and industrial use of pectinase[42]

9.Disadvantages of pectin lyase

Low yields: Pectin lyase yields from agricultural waste may be lower than those from other sources, including fungi. This is due to the fact that agricultural waste may not be a consistent substrate and that fermentation conditions might not be ideal for the production of pectin lyase.**[32]**

Impurities: Agro waste may contain impurities, including other enzymes and plant matter, which might increase the difficulty and cost of purifying pectin lyase.**[39]**

Variable quality: The type of agro waste used, the fermentation conditions, and the purification procedure can all affect the quality of pectin lyase produced from agricultural waste. Due to this, it may be challenging to generate a consistent product that satisfies the requirements of industrial users**[38]**.

Scalability: Increasing the production of pectin lyase from agricultural waste to commercial quantities can be difficult. This is due to the complexity and expense of the fermentation and purifying procedures. Environmental issues include: Growing and processing agricultural waste can have an adverse effect on the environment by causing water pollution and greenhouse gas emissions. Making sure that these effects are as little as possible is crucial. Some agro waste types may contain dangerous contaminants, such as germs and poisons, which raises questions about food safety. Prior to being utilized to create pectin lyase, it is critical to make sure that the agricultural waste is correctly handled and processed.**[38]**

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