MICROBIAL PECTINASES: A REVIEW ON MOLECULAR AND BIOTECHNOLOGICAL PERSPECTIVES

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ABSTRACT

Pectinases are upcoming enzymes in the biotechnological sector. This large group of enzymes involves in break down of pectic polysaccharides, the major component of middle lamella in plant cell wall. The pectin degrading enzymes are categorized according to their mode of action and release of degradative products. Various filamentous fungi, yeast, and bacteria are the common sources of microbial pectinases. Solid state fermentation is more preferable for extracellular microbial pectinases production over the submerged fermentation. A group of genes regulates the expression of pectinolytic enzymes. Mostly, all the enzymes share a common parallel β helix topology which consists of a single domain of parallel β-strands folded into a large right-handed cylinder. These enzymes are eco-friendly tools for industrial applications and being used in various sectors like wine, food, paper industry, waste paper recycling, fruit juice preparation, processing of tea–coffee, animal feed preparation.

Keywords: Pectin, pectinases, fermentation, crystal structure, extraction

INTRODUCTION

Enzymes are extremely efficient biocatalysts to perform all anabolic and catabolic reactions in living systems. They have efficient catalytic activity with definite mode of action, stereospecific binding, eco-friendly nature and low energy expenditure (**Bhardwaj *et al*., 2017**). Recently, several microbial enzymes have been extensively used in biotechnological processes as they are eco-friendly and economically sound for the industrial sector. Among the industrially important enzymes, pectinase has great importance in different industrial sectors like fruit and vegetable processing, extraction of vegetable oil, animal feed production, textile, recycling of wastepaper, wine and beverages production, tea and coffee processing (**Garg *et al*., 2016**). Pectinolytic enzymes are present in a complex enzymatic system which are capable to degrade the pectic substances (complex polysaccharide) those are mostly present in higher plants and microorganisms (**Jayani *et al*., 2005; Kittur, *et al*., 2003**). The commercial applications of pectinases were started from 1930s for the production of wines and fruit juices (**Ribeiro *et al*., 2010; Suneetha and Prathyusha, 2011; Oslen, 2013**). Applications of pectinases have increased with the passage of time and reached 20% in the enzyme worldwide market (**Hassan and Ali, 2016**). Plants, fungi, yeast and bacteria are the natural sources for pectinase production (**Ribeiro *et al*., 2010**).
Pectic substances are the versatile structural polysaccharides with high molecular weight and negative charges. They contain long galacturonic acid chains whose varying numbers of carboxyl groups are esterfied with methyl group. They are the major components of middle lamella of plant cell walls that occupies one-third of the dry weight of plant tissue (**Voragen *et al*., 2009; Khan *et al*., 2013**) and appear as cementing and lubricating agents in the cell walls. These components are responsible for giving the texture of fruits and vegetables during growth, and maturation (**Alkorta *et al*., 1998; Caffall and Mohnen, 2009**). Beside these, pectin substances are also related to several cellular functions of plants cells like growth and development, defense, seed hydration, ionic bonding, pH balance, cellular expansion, abscission of leaf and fruit development etc (**Lara-Marquez *et al*., 2011**). Pectins have several industrial applications, especially in the food and pharmaceutical sectors. The food industries use these components as gelling agent, nutritional fiber and also for removing of sugars and fats from low-calorie foods (**Sakai *et al*., 1993; Thakur *et al*., 1997**). In the pharmaceutical sectors, they are used as cholesterol reducing agent and also supplemented as lubricant in the preparation used for promoting intestinal movements. Moreover, these polysaccharides are also useful in drug delivery systems due to their low toxicity and durable activity without altering the

therapeutic effects of transporting drug (**Morris *et al*., 2010; Schols *et al*., 2009; Thakur *et al*., 1997**).
The major components of pectic substances are protopectin, pectins and pectic acids. The main chain of pectin contains α-1,4–D-galacturonan which is partially esterified with methyl group. Demethylated pectin is known as pectic acid (pectate) or polygalacturonic acid. Polygalacturonase cleaves the glycosidic linkages of polygalacturonic acid to produce mono-galacturonic acid. The inherent pectinase of plant cells is most important during the ripening of fruits as it starts degradation of middle lamella of plant's cell wall which leads to softening of fruits (**Khan *et al*., 2013**). Nowadays, pectinases are the integral part of various biotechnological applications especially in food industries for preparation fruit juices. The present review has focused on pectic substances, pectinolytic enzymes and their characteristics, and industrial applications.

PECTIC SUBSTANCES

Pectic substances are present in the primary cell wall in the form of calcium pectate and magnesium pectate. These are the major constituents of the middle lamellae which are a thin extracellular adhesive layer in between the walls of adjacent young cells and deposited in the early stages of cell growth to increase strength and support. The dicotyledonous plants contain approximately 35% pectin, 30% cellulose, 30% hemicellulose, and 5% protein in their primary cell walls while grasses contain 2–10% pectin (**Voragen *et al*., 2009**). Pectin regulates the various properties such as porosity, surface charge, pH, ion balance as well as ion transport in the cell wall (**McNeil *et al*., 1984**) and it also involves in plant defense by accumulating a broad spectrum anti-microbial component phytoalexin (**Jin and West, 1984**).
Pectins are the heterogeneous, branched and highly hydrated polysaccharides rich in D-galacturonic acid with negative charges. There are two major constituents of pectins, homogalacturonan (HG) and rhamnogalacturonan (RG); further, rhamnogalacturonan is subdivided into rhamnogalacturonan I (RGI) and rhamnogalacturonan II (RGII) (Fig.1). These three major pectic polysaccharides groups contain D-galacturonic acid to a greater or a lesser extent. The L-rhamnose residues may be attached to the main chain through its C-1 and C-2 atoms. Pectin in native form is interlined with other structural polysaccharides and proteins to form the water insoluble substance protopectin in unripe fruits. Synthesis of protopectin starts from UDP-galacturonic acid. The site of synthesis is Golgi system of younger cells whose cell walls are continuously enlarging at the early stages of growth (**Karr, 1976**). During ripening, the fruit enzymes break the pectin backbone or side chains, resulting in a more soluble molecule (**Kashyap *et al*., 2001**). The homogalacturonan (HG) of pectic substances forms

a gel structure after cross-linking with calcium ions. The gelling properties mainly depends on the interaction between calcium ions and the unesterified carboxyl groups of pectin; moreover, the other determining factors like temperature, pH, types of pectin, degree of esterification and acetylation, presence of sugar and other solutes are also important (**Pedrolli *et al*., 2009).**

Homogalacturonan (HG)

Homogalacturonan is a linear homopolymer of D-galacturonic acid residues attached through α-(1-4) glycosidic bonds and contains as many as 200 galacturonic acid units with a length of about 100 nm long. The acetyl esterification and methyl esterification of D-galacturonic acid can also be done at C-2 or C-3 position and C-6 position respectively (Fig.1). The methyl esterification determines physical properties of pectin and also sensitive to Ca^{2+} cross-linking. According to esterification level the molecules are classified into three groups: a) pectin, in which 75% of its carboxyl groups are methylated; b) pectinic acid which carries less than 75 % methylated carboxyl groups; c) pectic acid or polygalacturonic acid, which does not carry any methyl group at the carboxylic end. (**Jayani *et al*., 2005**).

Xylogalacturonan

Xylogalacturonan (XGA) is a substitute form of homogalacturonan, where β-D-xylose is present as single unit side chains through 1→3 linkages (Fig. 1) and it is commonly present in fruits and seeds (reproductive tissues). A varying degree of xylosidation had been observed in different fruits (25% in watermelon and 75% in apple) (**Albersheim *et al*., 1996; Le *et al*., 2001**).

Rhamnogalacturonan I (RG I)

RG I is a heteropolysaccharide, constructed by repeating disaccharide of rhamnose and galacturonic acid. The repeating disaccharide unit is linked through (1→2)α-L-rhamnose (1→4)α-D-galacturonic acid system. The Galacturonic acid residues of RGI are presumably not methyl esterified, but acetyl group can be present at the position of O-2 and/or O-3 of the GalA residues (Fig. 1). The neutral sugars such as galactose, arabinose, and xylose are linked with galacturonic acid residues and rhamnosyl residues at C-4 carbon as side chains (**Caffall and Mohnen, 2009; Willats, 2006**).

Rhamnogalacturonan II (RG II)

RGII is a homogalacturonan chain and mostly present in the plants as conserved structure. It bears four complex side chains which are attached to the galacturonic residues (**Willats, 2006**). It has the richest diversity of sugars and linkage characteristics. The unusual sugars are apiose, aceric acid (3-C′-carboxy-5-deoxy-L-xylse), 2-O-methyl fucose, 2-O-methyl xylose, 3-deoxy-D-manno-2-octulosonic acid (KDO), 3-deoxy-D-lyxo-2-heptulosonic acid (DHA) (Fig.1). These side chains are linked to a HG fragment of approximately nine GalA residues, of which some are methyl-esterified. RGII can form borate–diol ester in presence of Boron which can crosslink two HG molecules (**Ridley *et al*., 2001; O'Neill *et al*., 2001;Ishii and Matsunaga 2001**).

Protopectin

Protopectins are the heterogeneous mixture of pectic substances. They are present as water insoluble component in intact tissue.

PECTINASE PRODUCING ORGANISMS

Pectinolytic enzymes are abundantly present in nature. They are associated to metabolism of cell wall during cell growth, fruit ripening, abscission, senescence and plant pathogenesis (**Gaffe *et al*., 1997; Dorokhov *et al*., 1999**). Several organisms including bacteria, fungi, yeasts, insects, nematodes, protozoan and plants itself are able to produce these enzymes, but microbial pectinases are intrinsically most important as they are involved in plant-pathogenesis, plant-microbe interaction, and degradation of dead plant material (**Lang and Domenburg, 2000**). Pectinases are abundantly produced by saprophytic fungi, plant pathogenic fungi and several bacteria (**Gummadi and Panda, 2003; Hasunuma *et al*., 2003**). These microbial pectolytic enzymes show different mode of action and biochemical characteristics (**Favela-Torres *et al*., 2005; Gummadi and Panda, 2003**). Bacteria primarily produce alkaline pectinases while, fungi synthesize acid pectinases (**Hoondal *et al*., 2002; Jayani *et al*., 2005; Kashyap *et al*., 2001**).

Currently, there are several reports on importance of pectinolytic bacteria. However, the first report on pectin degradation by the bacterium *Erwinia* sp. was elucidated by **Elyrod (1942)**. Later, it was observed that many bacteria like *Erwinia, Yersinia* and *Klebsiella* sp. (**Chatterjee *et al*., 1979**), *Pseudomonas fluorescens* (**Zucker *et al*., 1972**) *Erwinia carotovora* (**McMillan *et al*., 1992; Heikinheimo *et al*., 1995**), *Xanthomonas campestris* (**Liao *et al*., 1996**), *Lactobacillus lactis* subsp. *Cremoris* (**Karam and Belarbi, 1995**), *Pseudomonas*

solanacearum (**Schell *et al*., 1994**), *Lachnospira pectinoschiza* (**Cornick *et al*., 1994**) produce inducible extracellular pectinolytic enzymes. Many fungal species are able to degrade pectin by secreting extracellular pectinases. The most common pectinolytic fungi are *Aspergillus niger, Aspergillus flavus, Aspergillus terreus, Penicillium chrysogenum, Fusarium moniliforme, Alternaria alternata, Cladosporium cladosporioids* and *Trichoderma reesei* (**Saranraj and Naidu, 2014**), *Alternaria citri* (**Isshiki *et al*., 2001; Juge, 2006**), *Rhodotorula* sp. (**Libkind *et al*., 2004**), *Phytophthora infestans* (**Forster, 1988; Sharma *et al*., 2013b**), *Saccharomyces cerevisiae* (**Gainvors *et al*., 1994; Alimardani-Theuil *et al*., 2011**), *Aspergillus niger* (**Maldonado *et al*., 1994; Maldonaldo and Saad, 1998**), *Penicillium frequentans* (**Kawano *et al*.,1999**), *Penicillium occitanis* (**Hadj *et al*., 2002**), *Aspergillus japonicas* (**Semenova *et al*., 2003**). The genus *Aspergillus* (**Kester and Visser, 1990**) and *Penicillium* (**Ikotun, 1984**) are commonly used for large scale production of pectinolytic enzymes.

Several studies had indicated that microbial pectinases are produced in multiple forms which differ on their molecular mass and kinetic behaviour (**Devi and Rao, 1996; Minjares-Carranco *et al*., 1997; Sathya *et al*., 2003**). **Caprari *et al*., (**1993**)** reported that polygalacturonases had expressed from one gene or from different genes. They also reported that pathogenic fungus *Fusarium moniliforme* produces four endo-polygalacturonases from one gene, but the enzymes differ at the extent of glycosylation of the same polypeptide. Another report had shown that polygalacturonases of *Aspergillus niger* are encoded by a family of diverged genes. (**Pedrolli *et al*., 2009; Coutinho *et al*., 2003**). Pectinases like large group of enzymes are encoded by multigenic families which arise from gene duplication and are linked in tandem (**Carroll *et al*., 2005**). The expression of these enzymes have diverged routes (**Coutinho *et al*., 2003**). Normally, pectin like polysaccharide cannot enter the cell due to their high molecular weight; but the induction of pectic enzymes in microbial system occurs in alternative way. Initially, low levels of constitutive expression of pectinolytic enzymes takes place before induction. These enzymes appear outside the cell and attack the polymeric substrate leading to release of low molecular products which act as inducers (**Fraissinet-Tachet and Fevr, 1996; Cooper, 1983**).

de Vries and his coworkers studied the expression of pectinolytic genes in *Aspergillus niger* (**de Vries, 2002**); the expression profile of 26 genes of these enzymes was studied under 16 different growth conditions. All genes were expressed in presence of D-galacturonic acid or a metabolite derived from it. **Benen *et al*., (**1996**)** reported that CCCTGA box is present at the promoters of many pectinolytic genes and might be involved in pectinolytic gene expression. Among the 26 pectinolytic genes, 14 genes contain CCCTGA box (**de Vries, 2002**). According to the expression profile, the pectinolytic genes are clustered into different subset. *pgaI, pgaII, pgaB, pgaC, pgaD, pgaE, pelB, pelC, pelF, plyA* and *rhgA* genes are placed into subset I and they are encoding enzymes for breakdown of pectin main chain. Their expression depends on presence of D-galacturonic acid, polygalacturonate and sugar beet pectin, incubation time and the pH of the medium. Besides these carbon catabolite repressor protein (CreA) also involves in regulation of expression and elimination of the effects of CreA is inductive for the gene expression. The other two genes *rglA* and *rhgB* (subset II) and *pgaA, pgaX, pelD, pelA,* and *pmeA* (subset III) encode enzymes active on the pectin main chain. The enzymes of subset III appear first and play major role to initiate degradation of pectin. The genes of subset IV (*lacA, abnA, abfA,* and *abfB*) and subset V (*faeA* and *faeB*) are encoding enzymes active on the side chains of pectin (**de Vries, 2002**). Several fungal pectin lyase genes (*pel*A, *pel*B) had been isolated and characterized from *Aspergillus niger* (**Gysler *et al*., 1990; Kusters-van *et al*., 1991; Kusters-van *et al*., 1992**), *A. oryzae* (**Kitamoto *et al*., 2001**). Recently, **Lima *et al*., (2017)** reported that expression of pectinolytic genes is regulated by catabolic repression. In presence of easily available metabolites, CreA-like protein binds at the promoters of gene (5′-SYGGRG-3′) to inhibit the gene transcription (**Kiesenhofer, 2016**).

PRODUCTION OF PECTINOLYTIC ENZYMES

Microbial pectinase production has been carried out through submerged fermentation (SmF) and solid state fermentation (SSF) by using fungi (**Dinu *et al*., 2007; Castilho *et al*., 2000; Silva *et al*., 2002; Pedrolli *et al*., 2008**) and bacteria (**Jacob *et al*.,2008; Klug-Santner *et al*., 2006; Kashyap *et al*., 2000; Soriano *et al*., 2005; Soares *et al*., 2001**). SmF is technically easier than SSF and used for enzyme production from the early stage of biotechnological progress. This process is conducted in stirred tank reactors under aerobic conditions by the use of batch or fed batch systems. Traditionally, SmF was more popular, as the process was very easy to operate and had a greater controlling capacity for the growth parameters like pH, temperature; but, there are some disadvantages: i) large amount of water is essential to operate the fermentation process, ii) continuous agitation must be needed to provide sufficient oxygen which increases the energy consumption as well as operational cost, and iii) produces huge amount of effluents at post fermentive stage. In addition to, huge capital investment, high energy costs and large infrastructural requirements also make it impractical for industrial purpose in modern age. In contrast to SmF, SSF is the simple technique in which microbial growth occurs on moist solid substrates with negligible free water under aerobic conditions. The solid substrate provides support or sometimes both support and nutrition.
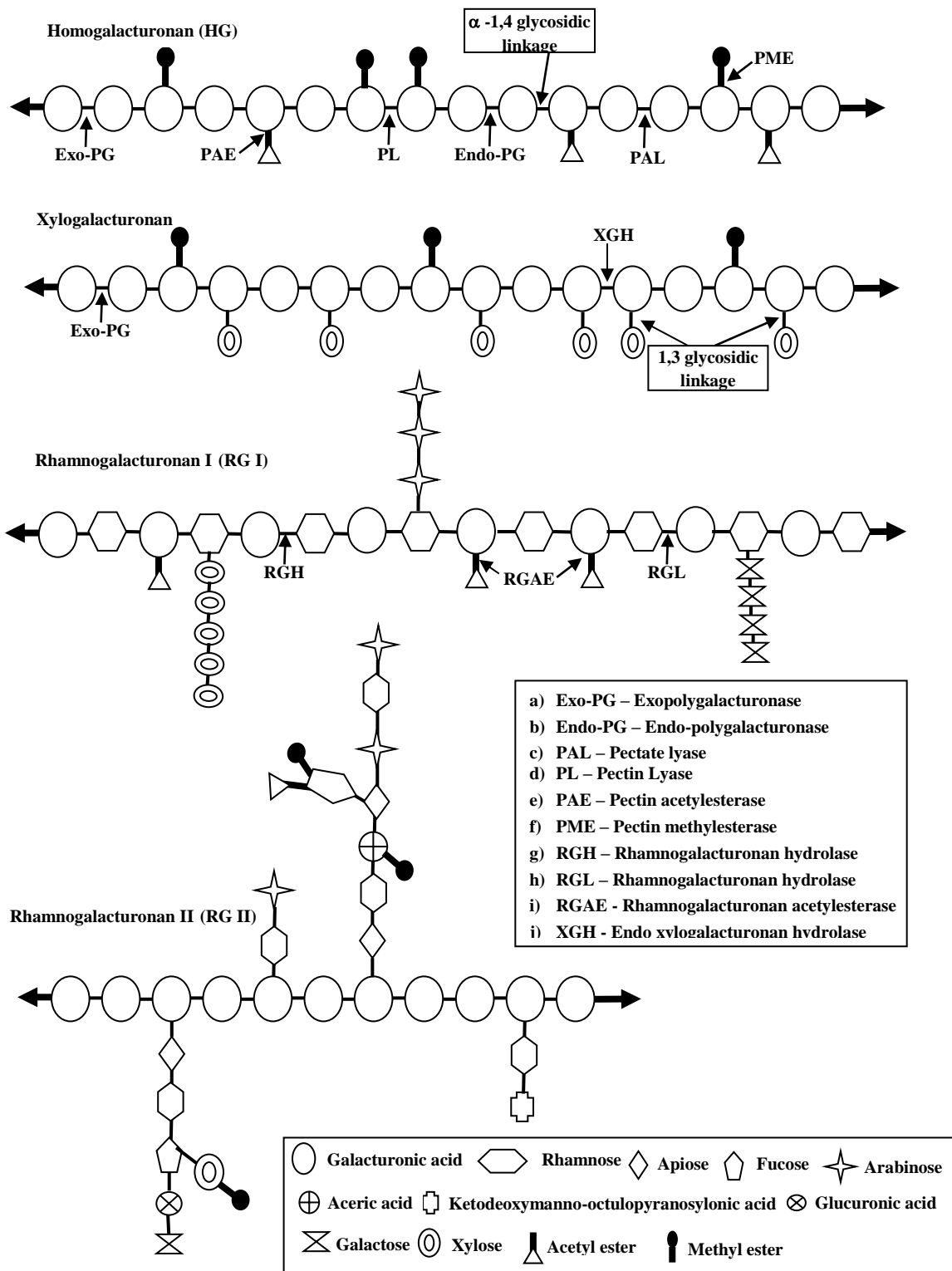
Figure 1 The schematic illustration of the basic architecture of pectin substances and the site for attack of pectinases concerned to the degradation of different types of pectin substances (**Voragen *et al*., 2009; Pedrolli *et al*., 2009**).

The major advantages of SSF include low capital investment, lower levels of catabolite repression and end product inhibition, low waste water output, higher productivity, greater enzyme yields and better product recovery (**Lonsane *et al*., 1985**; **Hölker and Lenz, 2005**).

About 50% of industrial enzymes come from filamentous fungi and yeast, and they are also widely used in submerged (SmF) and solid state fermentation (SSF) for pectinase production. Filamentous microorganisms are able to grow over the solid substrate. They have potential penetration power at their apical part which makes them more efficient to grow on the low moisture substrates in contrast to non-motile bacteria and yeast (**Smith and Aidoo, 1988**). Among the filamentous fungi *Aspergillus* gets most practical importance in SSF for pectinase production in commercial sectors. Several agricultural by products like cassava fibrous waste (**Budiatmen and Lonsane, 1987**), wheat bran (**Ghildyal *et al*., 1981**), apple

pomace (**Hours *et al*., 1988**) and citrus fruits wastes (**Garzon, and Hours, 1992**) were used as substrates for the SSF process. **Trejo-Hernanadez *et al*., (1991)** had conducted a comparative study to determine the yield of pectinase production from *Aspergillus niger* and reported that SSF is more productive than SmF.

Mehta *et al*., (1992) had isolated five strains of *Bacillus* which were able to produce large amount of polygalacturonase in submerged and semi - solid fermentation. **Sittidilokratna *et al*., (2007)** had isolated five strains *Erwinia* and seven strains of *Bacillus* for production of pectinases. *Erwinia chrysanthemi* strain N05 produced highest amount of polygalacturonase, while *Bacillus* sp. strain N10 gave maximum amount of pectate lyase and pectin lyase. Pectinase and polygalacturonase producing thermophilic filamentous fungal strain *Aspergillus fumigatus* was isolated by **Phutela *et al*., (2005)**. **Kutateladze *et al*., (2009)** reported that *Penicillium canescens* I-85, *Aspergillus niger*, *Trichoderma*

viride Ts-2 were able to produce maximum amount of pectinolytic enzymes in optimized culture conditions.

PECTOLYTIC ENZYMES

Pectinolytic enzymes or pectic enzymes or pectinases belong to hydrolases, lyases and esterases family which cleave the pectic substances through depolymerization and deesterification reactions. Table 1 shows the classification of different pectolytic enzymes.

Table 1 Different types of pectinases, their mode of action and reaction products (adopted from **Jayani *et al*., 2005; Garg *et al*., 2016).**

Types of enzyme	Common name	E.C. No.	Substrate	Mode of action	Product	Assay methods
De-esterifying enzymes						
Polymethyl galacturonate esterase (PMGE)	Pectin esterase	3.1.1.11	Pectin	Hydrolysis Random attack to cleave the methyl ester group of galacturonate unit	Pectic acid + methanol	Estimation of methanol by spectrometric method.
Pectin acetyl esterase	Pectin esterase	3.1.1.6	Pectin	Hydrolysis Random attack to cleave the acetyl ester group of galacturonate unit	Pectic acid + ethanol	Estimation of pNP spectrophotometrically by using pNP-acetyl substrate.
De-polymerising enzymes						
(a) Hydrolases						
(i) Polygalacturonases (PG)—Catalyzes the hydrolytic reaction of α-1,4-glycosidic bond in pectic acid						
Endopolygalacturonase (Endo-PG)	Polygalacturonase	3.2.1.15	Pectic acid	Hydrolysis Randomly attacks the pectic acid	Oligogalacturonates	Estimation of reducing sugar by using 3,5-dinitrosalicylate reagent or arsenomolybdate –copper reagent.
Exopolygalacturonase 1 (Exo-PG1)	Polygalacturonase	3.2.1.67	Pectic acid	Hydrolysis Starts bond breakdown from the terminal site of the non-reducing end of polygalacturonic acid	Mono-galacturonates	
Exopolygalacturonase 2 (Exo-PG2) (Exopolygalacturonan-digalacturono hydrolase)	Polygalacturonase	3.2.1.82	Pectate	Hydrolysis Penultimate attack	Di-galacturonates	
(ii) Polymethylgalacturonases (PMG)—Catalyses the hydrolytic reaction of α-1,4-glycosidic bond in pectin.						
Endo-PMG	Pectin hydrolase		Pectin	Random attack	Oligo methyl-galacturonates	The product is analyzed by paper chromatopraphy
Exo-PMG	Pectin hydrolase		Pectin	Attacks the bond from the terminal site of the non-reducing end of pectin	Methyl mono-galacturonate	
(b) Lyases						
(i) Polygalacturonate Lyase (PGL)—Catalyses the breakdown of α-1,4-glycosidic bond in pectic acid by trans-elimination and forms unsaturated galacturonates.						
Poly-(1-4)-α-D-galactosiduronate lyase (Endo-PGL)	Pectate lyase	4.2.2.2	Pectic acid /Pectate	Transelimination Random cleavage	Unsaturated oligo-galacturonates	Double bonds of the unsaturated products at the non-reducing ends can be detected to measure the increase in absorbance at 235 nm.
Poly-(1-4)-α-D-galactosiduronate exolyase (Exo-PGL)	Pectate lyase	4.2.2.9	Pectic acid /Pectate	Transelimination Cleavage of penultimate bonds from non-reducing end	Unsaturated di-galacturonates	
Oligo-D-galactosiduronate lyase	Pectate lyase	4.2.2.6	Oligo-galacturonate	Terminal cleavage	Unsaturated mono-galacturonates	Estimation of reducing group can also be possible by using 3,5-dinitrosalicylate reagent or arsenomolybdate –copper reagent.
(ii) Polymethylgalacturonate Lyase (PMGL)—Catalyses breakdown of α-1,4-glycosidic bond in pectin by trans-elimination and forms unsaturated methyl galacturonates from the non-reducing end.						
Endopolymethyl-D-galactosiduronate lyase (Endo -PMGL)	Pectin lyase	4.2.2.10	Pectin	Transelimination Random attack	Unsaturated methyl oligo-galacturonates	Same as Pectate lyase
Exopolymethyl-D-galactosiduronate lyase (Exo-PMGL)	Pectin lyase		Pectin	Terminal attack	Unsaturated methyl mono-galacturonates	

Act on Rhamnogalacturonan

Rhamnogalacturonan hydrolases	Rhamnogalacturonases	3.2.1.171; 3.2.1.173; 3.2.1.174	Rhamnogalact uronan chain	Hydrolysis	Oligogalacturonat es	Estimation of reducing sugar by using 3,5-dinitrosalicylate reagent or arsenomolybdate –copper reagent.
Rhamnogalacturonan lyases	Rhamnogalacturonan lyases	4.2.2.23; 4.2.2.24;	Rhamnogalact uronan chain	Act through random transelimination	Unsaturated galacturonate at nonreducing end of one oligomer and rhamnose as a reducing end residue of second oligomer	Double bonds of the unsaturated products at the non-reducing ends can be detected to measure the increase in absorbance at 235 nm.
Rhamnogalacturonan rhamnohydrolases (RGRH)		3.2.1.40	Rhamnogalact uronan chain	Hydrolysis from nonreducing end through exo-acting fashion	Rhamnose from terminal end	Estimation of Rhamnose by using cysteine hydrochloride and sulfuric acid reagent.
Rhamnogalcturonan Galacturonohydrolase (RGGH)			Rhamnogalact uronan chain	Hydrolysis from nonreducing end	Monogalacturonat e	Same as rhamnogalacturo nases
Xylogalacturonan hydrolase (XGH) Endo-acting		3.2.1.-	Xylose substituted galacturonan	Hydrolysis of α-1,4-D linkages through endo-acting fashion	Xylose-galacturonate dimers	

Protopectinases

Protopectinases were originally named by **Brinton *et al*., (1927)**.which cleave insoluble protopectin into highly polymerized soluble pectin (**Jayani *et al*., 2005**). The activity of protopectinase is determined by estimating the amount of pectin related material released from protopectin through carbazole sulphuric acid method. They are divided into two groups: type A: which reacts with the polygalacturonic acid region of protopectin, and type B: which reacts with the polysaccharide chains that is present in between polygalacturonic acid chain and cell wall constituents (**Sakai *et al*., 1993**). Microbial A type protopectinase is divided into different subtypes like protopectinase L, S and F. Their molecular weight is 30 kD; but F-type protopectinase is an acidic protein, while S and L-type protopectinases are basic proteins. On the other side, type B protopectinase is classified into B, C and T. The molecular weights of protopectinase B, C and T are 45, 30, and 55 kD respectively. The isoelectric point (pI) of protopectinase B and C are approximately 9.0 and protopectinase T has 8.1 (**Hassan and Ali, 2016**).

Pectin Methyl Esterases (PME) (EC 3.1.1.11)

Pectin methyl esterase or pectin esterase releases methyl group from 6-carboxyl group of galacturonic acid of pectin backbone and ultimately generates a non-methylaed galacturonate unit (pectic acid) and methanol (**Cosgrove, 1997**). It acts before the activity of polygalacturonases and pectate lyases because they only act on non-esterified substrates (**Kashyap *et al*., 2001**). Pectin methyl esterase is primarily produced by plants such as banana, citrus fruits and tomato and also by bacteria and fungi (**Hasunuma *et al*., 2003**).

Pectin Acetyl Esterases (PAE) (EC 3.1.1.1)

It is very similar to pectin methyl esterase and hydrolytically cleaves the esterified acetyl group of pectin, produces pectic acid and acetate (**Shevchik *et al*., 1997**).

Polymethyl galacturonases (PMG)

Basically, this enzyme belongs to hydrolase which hydrolytically cleaves the α-1,4-glycosidic bonds in highly esterified pectin backbone, forming 6-methyl-D-galacturonate (**Jayani *et al*., 2005**). The activity of the enzyme is measured by estimating the reducing sugars or by measuring the loss of viscosity of the substrate. Several species of *Aspergillus* and *Penicillium* are the rich sources of PMG.

Polygalacturonases (PG)

Polygalacturonase is under glycosyl-hydrolases family 28 which catalyzes α-1,4-glycosidic linkages in polygalacturonates (pectates), producing D-galacturonate (**Suneetha and Prathyusha, 2011**; **Coutinho and Henrissat, 1999**).

Polygalacturonases are mostly used in food industry for the preparation of jams, jellies, fruit juices and mixing of soluble dietary fiber (**Sharma *et al*., 2013a**). Polymethylgalacturonases and polygalacturonases (PMG and PG) can act in an endo-acting or exo-acting mode. Endo-acting PG mostly prefers non-esterified substrate and the activity decreases along with degree of methyl-esterification (**Parenicová *et al*., 2000**). Endo-PG (EC 3.2.1.15) and endo-PMG can act through random cleavage of substrate while, exo-acting PG (EC 3.2.1.67) and PMG starts hydrolysis of substrate from non-reducing end, produces monogalacturonate or digalacturonate in few cases (**Rombouts *et al*., 1980; Kashyap *et al*., 2001**). Fungi, bacteria and yeast are the major sources of endo PGs (**Sharma *et al*., 2012**) and the activity of PG can be determined as the same process of PMG.

Polygalacturonate lyase (PGL)

Pectate lyase is a Ca^{2+} dependent enzyme and cleaves glycosidic linkages through transelimination reaction. Ca^{2+} chelating agent like EDTA enables to inhibit the activity of PGL. Endo-PGL (EC 4.2.2.2) attacks α-1,4-glycosidic linkages of the substrate in random fashion, and exo-PGL (EC 4.2.2.9) starts substrate breakdown from non-reducing end by cleaving α-1,4-glycosidic linkages through sequential manner (**Rombouts *et al*., 1980; Pitt, 1988**). Microorganisms are the only sources of PGLs and produce Δ 4:5 unsaturated oligogalacturonates from substrate after enzymatic breakdon. PGLs show optimum pH in the region 6–10, which is much higher than other pectinases (**Truong *et al*., 2001; Dixit *et al*., 2004**).

Pectin Lyases (PL)

Pectin lyases (EC 4.2.2.10) are mainly endo-attacking and not strictly Ca^{2+} dependent but prefer cations for their action (**Pedrolli *et al*., 2009**). They degrade highly esterified pectic substances in a random manner, produce Δ 4:5 unsaturated oligomethylgalacturonates through transelimination reaction. Pectin lyase A from *Aspergillus niger* produces mono-, di-, tri- and tetragalacturonates and also generates unsaturated di-, tri- and tetragalacturonates from methyloligogalacturonates but not the monogalacturonates (**van Alebeek *et al*., 2002**). Fungal lyses are active in acidic and neutral medium; however, bacterial lyses show activity in alkaline medium.

Rhamnogalacturonan Rhamnohydrolases (RGRH)

Rhamnogalacturonan rhamnohydrolases are the group of enzymes comprising to RG rhamnohydrolase, rhamnogalacturonan α-L-rhamnopyranohydrolase or α-L-rhamnosidase (EC 3.2.1.40). They belong to glycosyl-hydrolase families 28, 78 and 106 (**Coutinho and Henrissat, 1999**). These enzyme hydrolyze rhamnogalacturonan chain at nonreducing end through exo-acting fashion and liberate terminal rhamnosyl residues attached (1→4) to α- galacturonosyl residues (**Mutter *et al*., 1994**).

Rhamnogalcturonan Galacturonohydrolase (RGGH)

RG galacturonohydrolase is a glycosyl hydrolase family 28 (**Coutinho and Henrissat, 1999**) which hydrolyses rhamnogalacturonan chain at nonreducing end producing monogalacturonate (**Mutter *et al*., 1998**).

Rhamnogalacturonan Hydrolases (RGH)

RG hydrolase randomly cleaves the α-D-1,4-GalA-α-L-1,2-Rha bond of rhamnogalacturonan chain, leaving Rha at the non-reducing end. The products are oligogalacturonates (**Mutter *et al*., 1998; Schols *et al*., 1990**).

Rhamnogalacturonan Lyases (RGL)

These enzymes belong to polysaccharides lyase families 4 and 11 (**Coutinho and Henrissat, 1999**). RG lyases are very similar to pectin lyases. They also act through random transelimination of the rhamnose-galcturonate linkage of rhamno-galacturonan chain. This enzyme cleaves the α-L-1,2-Rha-αD-1,4-GalA backbone leaving a 4-deoxy-β-L-threo-hex-4-enepyranosyluronic acid (unsaturated GalA) group at the non-reducing end. The hydrolyzing products are unsaturated galacturonate at nonreducing end of one oligomer and rhamnose as a reducing end residue of second oligomer (**Mutter *et al*., 1996; Voragen *et al*., 2009**).

Rhamnogalacturonan Acetylesterase

RG acetylesterase is under the carbohydrate esterase family 12 (**Coutinho and Henrissat, 1999**) which removes acetyl groups from rhamnogalacturonan chain.

Xylogalacturonan hydrolase

Xylogalacturonase also belongs to glycosyl-hydrolase family 28 (**Coutinho and Henrissat, 1999**). It acts on the α-1,4-D linkages of xylose-substituted galacturonan moieties in xylogalacturonan chain to produce xylose-galacturonate dimers after hydrolytic cleavage of glycosidic linkages.

PHYSICO-CHEMICAL CHARACTERIZATION OF PECTINOLYTIC ENZYMES

The efficacy of microbial enzymes depends on the various parameters like pH, temperature, incubation time, agitation, concentration of enzyme and substrate, presence of metal ion and inhibitors, viscosity of the medium, presence of emulsifier, and interaction between enzyme and substrate interface. Many researchers had described the characteristics, biochemical properties and mechanisms of action of microbial pectic enzymes and Table 2 shows some physicochemical properties of pectic enzymes produced by different microorganisms. The optimum pH and temperature of pectinases from different sources vary between 3.5-11 and 40-75 °C respectively (**Gummadi and Panda, 2003; Kashyap *et al*., 2001**).

Table 2 Physicochemical properties of some pectinases and their sources.

Types of enzyme	Source		Types of enzyme	Molecular mass (kD)	Optimum pH	Optimum temperature (°C)	References
	Fungi	Bacteria					
Polygalacturonases	*Aspergillus japonicus, Aspergillus niger, Aspergillus awamori, Saccharomyces cerevisiae*		Endo	38 – 63	3.0–5.5	30 – 45	**Hasunuma *et al*., 2003; Singh and Rao, 2002; Nagai *et al*., 2000; Corredig *et al*., 2000**
	Penicillium frequentans		Exo	63	5.0	50	**Favey *et al*., 1992**
	Fusarium oxysporum		Exo	38	11.0	69	**Pietro and Roncero, 1996**
		Bacillus sp KSM-P410	Exo	63	7.0	60	**Koboyashi *et al*., 2001**
		Bacillus licheniformis	Exo	38	11.0	69	**Singh *et al*., 1999**
Lyases	*Aspergillus japonicus, Penicillium italicum, Penicillium adametzii, P. citrinum, P. janthinellum*		PMGL		6.0–8.0	40 – 60	**Alana *et al*., 1990; Sapunova *et al*., 1995; Dinnella *et al*., 1994**
		Erwinia carotovora, Bacillus macerans, Yersinia enterocolitica, Bacillus sp. TS4	PGL	36– 55	8.0–11.0	50 – 70	**Miyazaki, 1991; Fayyaz *et al*., 1993; Singh *et al*., 1999; Takao *et al*., 2000; Kashyap *et al*., 2000**
Pectinesterases		*Erwinia chrysanthemi B341, E. chrysanthemi 3604*		37	5.0–9.0	50	**Pitkänen *et al*., 1992; Maldonaldo and Saad, 1998**
	Aspergillus japonicus			47	4.0–5.5		**Hasunuma *et al*., 2003**

Polygalacturonases (PG)

Polygalacturonases (endo PGs and Exo PGs) of different microbial sources show different physicochemical properties. Normally, the optimum pH and temperature of PG range from 3.5–5.5 and 30–50 ºC respectively. While, PG obtained from *B. licheniformis* and *F.oxysporum f. sp. Lycopersci* is active in highly alkaline pH (optimum pH 11). The endo PGs exist in different forms; their molecular weights, pI, optimum pH and temperature vary in between 30–80 kD, 3.8 -7.6, 2.5–6.0 and 30–50 °C respectively (**Singh and Appu-Rao, 1989; Takao *et al*., 2000**). The endo PGs are the inverting glycosidases which invert the

anomeric structure of the products and rapid loss of viscosity of substrate has also been observed during hydrolyzing reaction. Similarly, Exo PGs of *A. niger*, *Erwinia* sp. and some plants (peaches, citrus and apples) have shown the molecular weight and pI in between 30-50 kD and 4.0-6.0 respectively (**Alonso *et al*., 2003; Pathak and Sanwal, 1998**). The mode of action of fungal exo-PGs and bacterial exo-PG are quite different. Fungal exo-PGs give monogalacturonic acid as the end product from non reducing end of polygalacturonic acid; whereas, bacterial exo-PG produces di-galacturonate from the same reaction (**Sakai *et al*., 1993**).

The mechanism of hydrolytic reaction has been done through a general acid base catalysis system. During reaction, acid catalyst donates a proton to the glycosidic oxygen, and then the base part starts the nucleophilic attack on the anomeric carbon of the galacturonate moiety (**Shimizu et al., 2002**). The study of site-directed mutagenesis had revealed that the substitution of charged amino acids His195, Arg226, Lys228, and Tyr262 in endo PG II of *A. niger* drastically lowers the substrate affinity by increasing the K_m value more than tenfold (**Armand et al., 2000; Pages et al., 2000**). Therefore, the tight substrate binding is maintained by the electrostatic interactions between the free carboxyl group of substrate and the basic amino acid residues of the active site of enzyme. The another probability is that the endo PGs enable to cleave only the non-esterified (methyl group) free polygalacturonate substrate (**Shimizu et al., 2002**). Subsequently, the replacement of Asp173 in the active site of enzyme slightly increases K_m value (two fold), but significantly decreases the K_{cat} value (**Armand et al., 2000**). This finding has concluded that Asp173 is responsible for catalysis and acts as a general acid catalyst that donates a proton to the glycosidic oxygen.

Polymethyl galacturonase (PMG)

Highly methylated pectin (95 %) is the best substrate for polymethyl galacturonase and its optimum pH lies between 4.0-5.0 (**Schnitzhofer et al., 2007**). Previously, **Sathya et al., (1998)** reported that *A. niger* produces highly acidic PMG (optimum pH 2.3). PMG acts through β-elimination mechanism and produces unsaturated oligogalacturonides from pectin polymers.

Polygalacturonate lyase (PGL)

The molecular mass of PGL varies between 30-50 kD (**Sharma et al., 2012**). Most of the PGL is active in alkaline pH range. The PGL from *Erwinia* sp. and *Bacillus licheniformis* showed highest activity in the range of pH 8.0-10.0 and 6.0-11.0 respectively. The enzymatic action is very similar in all the PGL families. Generally, PGL randomly cuts pectates by β-elimination process and generates a trimeric end-product, carries an unsaturation unit at Δ^4 position in the galacturonosyl residue at the non-reducing end (**Petersen et al., 1997**). The reaction occurs in three steps: (a) calcium ion and asparagine help to neutralize the carboxyl group nearby to the attacking glycosidic bond, (b) abstraction of the C5 proton is mediated by arginine, and (c) transfer of the proton to the glycosidic oxygen, resultant is formation of a double bond in between C4 and C5 at the non-reducing end of the product (**Sharma et al., 2012**). **Pickersgill et al., (1994) and Herron et al., (2003)** had revealed that Ca^{2+} is essential for in-vitro activity of Pel C. The Ca^{2+} ions create link between oligosaccharide, and protein and it also appears to the nearby uronic acid moieties within a single pectate chain. In this system, position of Ca^{2+} is quite different from the position of Ca^{2+} in PGA, where Ca^{2+} ions help to link PGA helices together (**Walkinshaw and Arnott, 1981a, b; Jayani et al., 2005**).

Pectin lyase (PL)

The molecular weight of PL lies in between 30–40 kD (**Soriano et al., 2005; Hayashi et al., 1997**), having isoelectric point within the range of 3.5. Generally, PL is active in acidic pH range of 4.0–7.0, but there are some reports about the activity of PL in alkaline conditions (**Sakiyama et al., 2001; Moharib et al., 2000**). The optimum pH and temperature of PL from *A. niger* were 4.8 and 35 °C respectively (**Sathya et al., 1999**). The K_m value for PL depends on the substrate specification and it remains within the range of 0.1-5 mg/ml (**Sakiyama et al., 2001; Moharib et al., 2000**). The mechanism of catalysis is very similar to PGLs and this also occurs via β-elimination; however, substrate specification and cofactor requirement are not the same as PGL. The activity of PL is Ca^{2+}

independent and highly methylated pectin is the best substrate in comparison with PGLs which act on the non-methylated substrate in presence of calcium ions. Though, the crystal structures of PGL and PL are structurally almost identical having β-helix fold; they show some essential divergences in their activity. The loop present in the active site of PL is much longer than PGL and consists of two β-strands forming an antiparallel β-sheet. The cleft of the putative active site of PL contains four tryptophans and three tyrosines. These aromatic amino acids contribute a major role in the architecture of the active site (**Sharma et al., 2012**).

Pectin esterase or pectin methyl esterase (PE)

Pectin methyl esterase starts de-esterification by hydrolyzing the ester bond of methylated α-(1-4)-linked D-galacturonosyl units and the end products are negatively charged galacturonosyl polymer and methanol. Most of the microbial PEs had shown their molecular weight in between 30-50 kD (**Hadj et al., 2002; Christensen et al., 2002**) and pI varies from 4.0-8.0. Except the PE from *Erwinia* (alkaline PE), the optimum pH of others PEs varies in between 4.0-7.0. The optimum temperature of most PE varies in between 40–60 °C (**Sharma et al., 2012**). The molecular structure of PE reveals that the location of active site and substrate binding cleft are very similar to PGL and PL. In this manner, the central portion of the substrate binding cleft is surrounded by several aromatic amino acids, whereas Asp136 and Asp157 are present in the centre of the active site (**Sharma et al., 2012**). **Johansson et al., (2002)** had shown the role of Asp136 and Asp157 for the action of PE. Asp157 exerts the nucleophilic attack on the carboxy methyl carbonyl carbon and Asp136 may act proton donor (acid) in the first cleavage step during releases of methanol. At the later stage, Asp136 extracts hydrogen from an incoming water molecule and cleaves the association between enzyme-substrate complexe for reactivation of the active site of the enzyme.

THE MOLECULAR BASIS OF STRUCTURE FUNCTION RELATIONSHIP

The molecular basis of enzymatic action had been elucidated after the determination of three-dimensional structures of pectinases. **Keen et al., (1984)** first cloned the *pelC* (pectate lyase C) gene of *Erwinia chrysanthemi* in *Escherichia coli* and the crystal structure of PelC of *Erwinia chrysanthemi* was elucidated by **Yoder et al., (1993)**. Later, the structural configuration of the pectinases family had subsequently revealed. These pectinases were pectate lyase A (PelA) (**Thomas et al., 2002**) and PelE (**Lietzke et al., 1994**) of *Erwinia chrysanthemi*, Pel of *Bacillus subtilis* (BsPel) (**Pickersgill et al., 1994**), high alkaline pectate lyase of *Bacillus* sp. (**Akita et al., 2000**), pectin lyases A (PLA) (**van Alebeek et al., 2002**) and pectin lyases B (PLB) (**Vitali et al., 1998**) from *Aspergillus niger*, polygalacturonase of *Erwinia carotovora* (**Pickersgill et al., 1998**), endo-polygalacturonase I and II from *A. niger* (**Van Pouderoyen et al., 2003; Van Santen et al., 1999**), polygalacturonase of *Aspergillus aculeatus* (**Cho et al., 2001**), endopolygalacturonase of *Fusarium moniliforme* (**Federici et al., 2001**); pectin methylesterases from *Daucus carota* (**Johansson et al., 2002**), PemA from *E. chrysanthemi* (**Di Matteo et al., 2005**) and rhamnogalaturonase A of *Aspergillus aculeatus*. (**Petersen et al., 1997**). Another report had suggested that the pectin lyase A of two strain of *A. niger*, N400 and 4M-147 has some dissimilarity in the loop conformation, made by amino acid residues 182–187 (**Mayans et al., 1997**). Figure 2 shows the ribbon diagram of few pectinases from various sources.

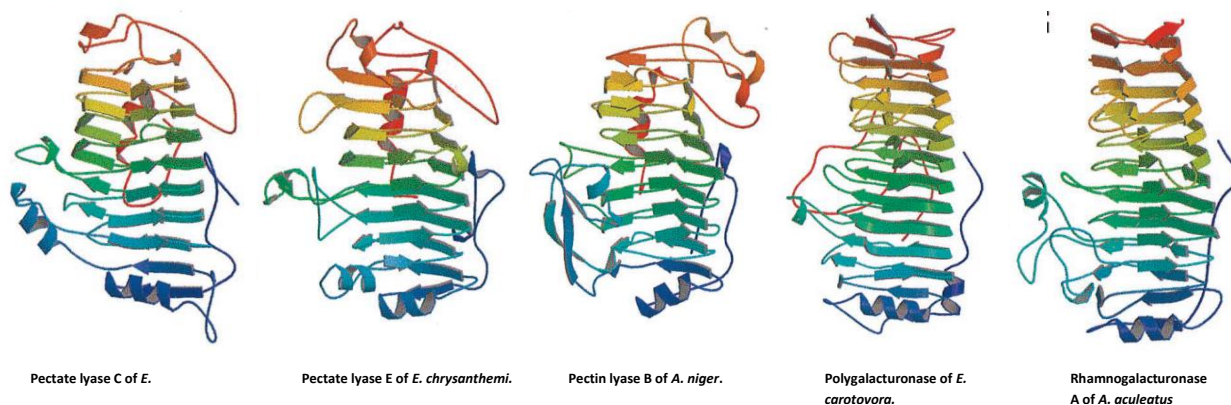| Pectate lyase C of *E.* | Pectate lyase E of *E. chrysanthemi.* | Pectin lyase B of *A. niger.* | Polygalacturonase of *E. carotovora.* | Rhamnogalacturonase A of *A. aculeatus* |

Figure 2 Structural presentation of five different pectinases those are configured by parallel β helix motif. The major portions of secondary structure of these enzymes are constructed by β strands, and the coils represent α helices (Figure adopted from **Herron et al., 2000).**

PNL, PL, PG, and PE are the predominant pectinases and their sequences are available in GeneBank of NCBI. The multiple sequence alignment study can give the idea about diversity and evolutionary relationship of the pectinases from different sources and also provide the information to design the global primer for PCR application and enormous expression.

In silico study had revealed that the similarity in base sequences is present in the different pectinases. The enzymes were classified in different groups on the basis of source organisms and mode of action (**Yadav *et al*., 2009**). This study also indicated that the unique similar type domains are available in pectin lyase, pectate lyase and also in pectin esterase. While polygalacturonases have another unique domain; they belong to glyco-hydrolases 28. The multiple sequence alignment study of pectinases had shown a homology at the residues from 317 to 440 and from 553 to 676. **Yadav *et al*., (2009)** also observed that a conserved protein sequences from residues 553 to 679 and from 680 to 806 are present in 48 protein sequences of pectin lyases. The mechanism of catalysis of the hydrolases and the lyases are completely different but in both cases, the substrate binding sites lie in a cleft which appears as the exterior part of the parallel β-helix (**Herron *et al*., 2000**). Previously, **Xiao *et al*., (2012)** reported that the gene of pectate lyase of *Bacillus subtilis* 521 encoded 420 amino acids with 37% negatively charged residues and 39% positively charged residues. The proteomics study of pectate lyase from *Bacillus subtilis* 521 had shown high homology to those from other bacteria. The catalytic domains are present in two positions 45-QTDASNGANYITMS-258, 296-VQRAPRVRFGQVHVYN -312 and the catalytic activity is Ca^{+2} dependent. The gene sequence of pectate lyase had shown 26-82% similarity with other bacteria (*B. licheniformis* and *B. amyloliquefacien*).

Pectate lyases

The study of three dimensional structure of pectinolytic enzymes had indicated that there is a common structural configuration among the enzymes. The crystal structures of pectate lyase C, pectate lyase E and pectate lyase of *Bacillus subtilis* were comprised by a right-handed parallel β-helix and a major loop region (**Mayans *et al*., 1997**). **Henrissat *et al*., (1995)** had indicated that Asp184 and Arg279 are invariant amino acid residues in the pectate and pectin lyase family. Other conserved amino acids *Val*-Trp-*Ile*-Asp-His (*Val*, *Ile* substitutable) are present at the opposite side of the parallel β helix. The presence and importance of right-handed β- helix in various pectinolytic enzymes like pectin lyase B, pectate lyase C, pectate lyase E and bacterial pectate lyase had been reported by several works (**Yadav *et al*., 2009**). In addition to the β-helix structure, uncommon configuration like (α/α) toroid and (α/α) barrel structure had been observed in *Cellvibrio japonicus* and *Yersinia enterocolitica* separately (**Gummadi *et al*., 2003; Natada *et al*., 2000**).

An early study indicated that pectate lyase C is composed of 353 amino acids, with two disulfide bonds (**Keen *et al*., 1984**) and Ca^{2+} is necessary for its activity. The detailed structure of pectate lyase C from *Erwinia chrysanthemi* had revealed that there are eight coils in the right-handed parallel beta helix and each comprises three β strands, joined by three turns. Within this topology, three parallel β sheets are formed due to staking of the coils and the structure is stabilized by an extensive network of inter-strand hydrogen bonds. The core of the parallel β helix contains hydrophobic, aromatic, or polar amino acids. These amino acids are oriented toward the interior, make arrangement in a row with amino acids of neighboring coils; thus, form long ladders. The amino acids of exterior part are randomly oriented and form loops of varying length which protrude from the central core (**Herron *et al*., 2000**). Another isoenzyme of pectate lyase C is pectate lyase E which shares 22% sequence identity and contains a single disulfide bond. The later is associated to a different subfamily of pectate lyases. In compare to PelC, the charged amino acids are randomly distributed on the surface of pectate lyase E. The sequence based study of 14 extracellular pectate lyases, 7 pectin lyases had revealed that there are 10 invariant amino acids which are arranged in a cluster around the active site and out of them, 5 amino acids have been associated in catalysis. The catalytically important amino acids are Asp-131, Asp-144, His-145, Thr-206, and Arg-218; while, other five amino acids (Gly-6, Gly-12, Gly-13, Trp-142, and Pro-220) are chemically inert. Moreover, there are two distinct clusters of amino acids; Asp-131, Arg-218, and Pro-220 are present in the region of the Ca^{2+} binding site which is the part of the active site, other seven amino acids are distributed on the opposite side of the parallel β helix (**Herron *et al*., 2000; Henrissat *et al*., 1995**). Site-directed mutagenesis study had indicated that alteration of amino acids around the Ca^{2+} binding site and Asp-131 and Arg-218 reduce the pectolytic activity (**Kita *et al*., 1996**).

Pectin lyases

Pectin lyases and pectate lyases have similar type of enzymatic activity but each enzyme recognizes a different substrate. The pectin lyase A from two strains of *Aspergillus niger*, N400 and 4M-147 contains 359 residues in their mature form (**Mayans *et al*., 1997**). They share common structural features with only 17% sequence identity. The protein sequences (from N terminus) of these two strains differ in nine amino acid substitutions: Glu12→Lys, Asp19→Ser, Val101→Thr,

Gly213→Ala, Cys248→Ala, Phe253→Trp, Ala289→Glu, Thr296→Ser and Cys317→Ser (from strain N400 to strain 4M-147 and number started from N terminal). The pectin lyase from strains N400 and 4M-147 folds into a large right-handed cylinder, made by parallel β-helix which is common structural configuration of various pectinolytic enzymes. The core of the cylinder is composed by seven to nine complete helical turns and each consists of three parallel β-strands with unique arrangement. In this topology, the strands of consecutive turns are stacked to form three parallel β-sheets called PB1 (contains eight β strands), PB2 and PB3 (contains nine β strands) which are stabilized by extensive network of inter-strand hydrogen bonds. PB1 and PB2 give an antiparallel β structure, while PB3 lies approximately at right angle to PB2 (**Mayans *et al*., 1997; Sharma *et al*., 2012**). There are three turns T1 (between PB1 and PB2), T2 (between PB2 and PB3) and T3 (between PB3 and PB1) within the β sheets. Among these, T1 is the β arch type and T2 is in the 90° direction to the polypeptide backbone (Fig. 3). These turns also contain asparagine ladder and the structure of T3 loop is hold up by two disulphide bridges, Cys63–Cys82 and Cys72–Cys206; while, T2 loop is preserved by a single disulphide bond (Cys302–Cys310). The N-terminal tail packs against PB2; whereas, a highly conserved amphipathic α helix is present in C-terminal tail which lies across PB3 and packs against the T2 turn. The maximum conserved part of PB2 and PB3 is in the asparagine ladder in T2 and the Val-Trp-Ile-Asp-His pattern in PB2. All these parts contribute to structural conservation of parallel β-helix structure; while, diversity is present around the active site. These findings indicate that the conserve tails support the evolutionary relationship between pectate and pectin lyases.
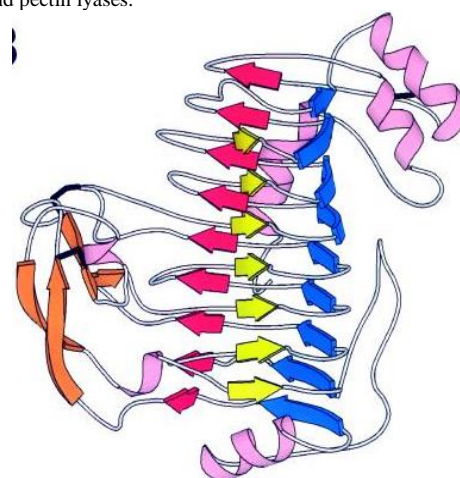

Figure 3 Secondary structure of pectin lyase B. Pink coils indicates the helices and arrows represent the β strands. Yellow arrows are given for PB1; PB2 is blue; and PB3 is red. The orange arrows indicate the antiparallel β structure within the first T3 turn and a short β strand within the third T3 loop. The thick, black lines are given to indicate disulfide bonds. This image was developed by MOLSCRIPT, adopted from (**Kraulis, 1991**).

Although, all the pectinolytic enzymes share the same parallel β helix topology, the mechanism of breakdown of pectin is different among the esterases, hydrolases and lyases. The substrate binding site of these enzymes has common sequence similarity and is present in the similar position within a cleft. This site is present at the exterior site of the parallel β-helix between one side of PB1 and the protruding loops (**Kita *et al*., 1996; Sharma *et al*., 2012**). While, the structural differences within the loops are associated to the catalytic activity and their properties. The substrate-binding site of pectate lyases are present between the long T3 loops and PB1 (**Henrissat,*et al*., 1995**). The cleft is dominated by aromatic residues, comprising four tryptophans (Trp66, Trp81, Trp151 and Trp212) and three tyrosines (Tyr85, Tyr211 and Tyr215). They form three pairs of residues (Trp81–Trp151, Trp66–Trp212 and Tyr211–Trp212) which are essential for the architecture of the binding cleft. The charged amino acids Asp154, Arg176 and Arg236 within the active site exert catalytic power (**Mayans *et al*., 1997**). Replacement of Trp62 with the aliphatic amino acids in egg white lysozyme through site directed mutagenesis studies had indicated that aromatic residues prefer binding with uncharged polysaccharides during reaction. Moreover, the substrate-binding region of pectate lyases abundantly carries charged acidic and basic amino acids for substrate recognition. In certain cases Ca^{2+} plays a vital role; for example, Ca^{2+} binds with three aspartate residues: Asp184, Asp223 and Asp227 at the bottom of the substrate-binding cleft of BsPel (*Bacillus subtilis* pectate lyase). Among these three amino acids, Asp184 and Asp227 are absolutely conserved, while Asp223 may be replaced by another acidic amino acid glutamate. In contrast, the pectin lyase A contains Arg176

similar to Asp223, and Val180 is equivalent to Asp227; while, Asp154 is comparable to Asp184 of pectate lyase and Arg236 (pectin lyase numbering) in binding cleft is preserved among the extracellular pectate and pectin lyases families. Thus, it is presuming that extracellular pectate and pectin lyases had been arisen from a common ancestral lyase. Generally beta elimination reaction is very similar to acid base catalysis and some time metal ions have vital role in activation. The carboxylate anion group at C6 position of pectate interacts with Ca^{2+} ion in pectate lyase and stabilizes the substrate binding, because Ca^{2+} binds with Asp223 which forms the environment equivalent to Arg176 in pectin lyase. The bound Ca^{2+}, forms an elongated ribbon of positive potential which is complementary to the negatively charged pectate substrate. Thus, the roles of Ca^{2+} in pectate lyase and the arginine in pectin lyase A are very similar in respect of stabilizing negatively charged substrates. The resultant is catalysis by pectate lyase is Ca^{2+} dependent but pectin lyase does not.

Polygalacturonases

Mukadam *et al*., (2010) reported that the length of gene of polygalacturonase of *Aspergillus* species ranges from 1107 to 2495 nucleotides. They had isolated polygalacturonase I from *Aspergillus niger* which contains 1101 nucleotides and 367 amino acids. The multiple sequence alignment of amino acid sequence of polygalacturonase enzyme from different fungal species like *A. niger* (XM 001389525; **Pel *et al*., 2007)** and *A. fumigatus* (XM 746347; **Nierman *et al*., 2005**) shares 96% and 76% similarity respectively.

All the polygalacturonases (endo or exo) are in glycoside hydrolase family 28 (GH28). The results of analysis of amino acid sequence of all the polygalacturonases from various sources have shown the four highly conserved regions (**Palanivelu, 2006**). The amino acid residues of these four motifs are NTD, G/QDD, G/SHG, RIK. Among these four regions, the two are catalytic regions (G/QDD and G/SHG) and the other two are substrate-binding regions. The distances (number of amino acids) between these conserved motifs are specifically maintained in most of the cases. The average amino acids between NTD→QDD, QDD→GHGMSIGS, GHGMSIGS→RIK are 21-22, 22-28, 30-40 respectively (Table 3). This precise distance among the highly conserved motifs may be essential for substrate binding and catalysis. **Palanivelu (2006)** also reported that acidic amino acid Asp and basic amino acid His are responsible for catalysis of glycosidic bond in polygalacturonates (pectates). Here, His acts as a proton donor and the Asp acts as a nucleophile and the reaction is very similar acid base catalysis (Fig. 4). This reaction mechanism is very similar to other glycosidases like levansucrases (**Chambert *et al*., 1976**), α-amylases (**Tao *et al*., 1987; van der Maarel *et al*., 2002**), and xylanases (**Jeffries, 1996**). Furthermore, the mutation at the His188 drastically affects the enzyme activity and indicates that the highly conserved His188 is essential in the active site. Alteration of a critical Asp212 by Glu or Asn of polygalacturonase from *Fusarium moniliforme* did not show any activity. Thus Asp212 acts as nucleophile instead of proton donor because replacement of Asp212 by Glu must be shown some catalytic activity.

Table 3 Conserved motifs and distance between motifs of polygalacturonases from various sources. Numbers of amino acids between the conserve motifs are minutely variable. (Sequences are taken from SWISSPROT/ TrEMBL database, adopted from **Palanivelu, 2006**).

| Name of the organism	Motif I (*Substrate binding site)	Distance between motif (number of amino acids)	Motif II (*Catalytic site)	Distance between motif (number of amino acids)	Motif III *Catalytic site)	Distance between motif (number of amino acids)	Motif IV (*Substrate binding site)
Bacteria							
***E. carotovora* P 26509**	[201]NTD	21	[222]GDD	28	[250]GHGMSIGS	30	[280]RIK
***X. campestris* Q8P582**	[243]NTD	22	[265]GDD	26	[291]THGISIGS	40	[331]RIK
***P. syringae* Q87Y49**	[293]NTD	22	[315]GDD	27	[342]GHGLSIGS	35	[377]RIK
Fungi							
***S. cerevisiae* (endo) P 47180**	[177]NTD	22	[199]QDD	22	[221]GHGISVGS	34	[255]RIK
***A. niger* (endo II) P 26214**	[178]NTD	22	[200]QDD	22	[222]GHGLSIGS	34	[256]RIK
***A. tubingensis* (exo) Q 0093**	[221]NTD	22	[243]GDD	23	[266]SHGISVGS	36	[302]RIK
***C. carbonum* (exo) Q 00359**	[227]NTD	22	[249]GDD	23	[272]SHGISVGS	36	[308]RIK
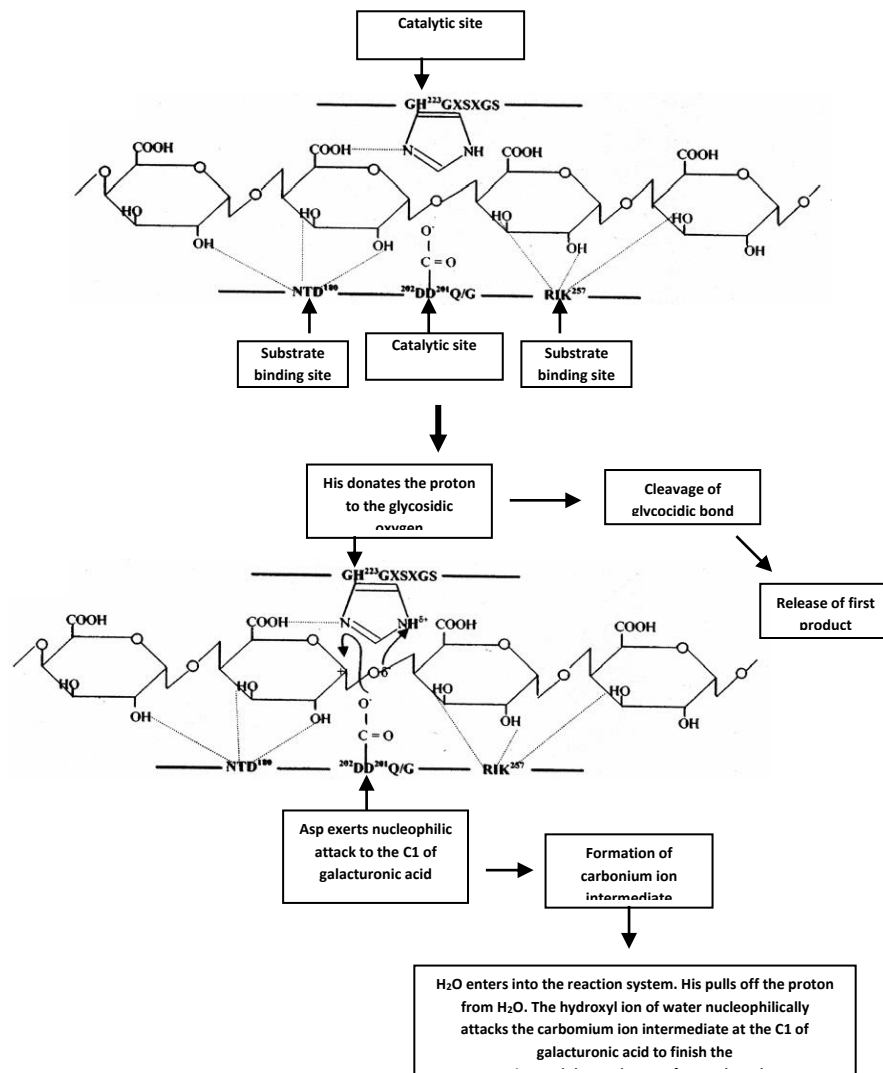
Figure 4. Mechanism of action of polygalacturonase from *A. niger*. The primary figure adopted from **Palanivelu, 2006** and modified.

The X-ray crystallographic study had indicated that His is present in the catalytic site and can act as a proton donor. Previously, **Schroter *et al.*, (1999)** stated that polygalacturonases act in acidic pH optima and a protonated His in the active site plays vital role. The presence of His in active site of polygalacturonase of *Aspergillus ustus* was also reported by **Rao *et al.*, (1996)**. **Caprari *et al.*, (1996)** had performed the site-directed mutagenesis study on *Fusarium moniliforme* and suggested that His234 is the critical amino acid in the polygalacturonases which is present in highly conserved GH234GXSIGS region. The replacement of His234 by another basic amino acid Lys totally stopped the catalytic function, as the pI of His and Lys are 7.59 and 9.74 respectively. Another study of site-directed mutagenesis for substitution of His223 by four different amino acids in endo-polygalacturonase II from *A. niger* (**Armand *et al.*, 2000**) completely decreases the substrate affinity as well as enzyme activity. Polygalacturonases from various organism *E. caratovora* (**Pickergill *et al.*, 1998**), *A. niger* (**van Santen *et al.*, 1999**) and *F. moniliforme* (**Federici *et al.*, 2001**) have also exhibited similar structural motifs (right- handed parallel β-helix) like pectin and pectate lyases. Moreover, the enzymes have two loop regions which form a tunnel like substrate-binding site and all the four conserved regions are present in this cleft site. The Asp of G/Q**D**D and His of G**H**GMSIGS region are located within 5.5 Å distance and optimally serve as a nucleophile and a proton donor, respectively (**Palanivelu, 2006**).

Pecti methylesterase (PME)

Pectin methylesterase (PME; E.C.3.1.1.11) belongs to family 8 carbohydrate esterase (CE8). The multiple sequence alignment study had revealed that there were some conserved and semi-conserved amino acid residues in the PME from various sources. Like other pectinolytic enzymes, PME is also comprised by right handed parallel β-helices and above 80% amino acid residues reside in most-favorable region of the Ramachandran plot (**Rajulapati *et al.*, 2018**).

APPLICATIONS OF MICROBIAL PECTINASES

Pectinases are the most useful industrial enzymes. They have been used in several industries for processing of fruits and vegetables, beverages and wine,

tea, coffee, textile material, and animal feed. Beside these, other arena of applications of these enzymes are plant fiber processing, saccharification of polysaccharide substrates, extraction of vegetable oil, degumming of plant bast fibers, bio-bleaching of kraft pulp, making of paper pulp, recycling of wastepaper, treatment of pectinacious waste in industrial effluents, and plant viruses purification (**Salazar and Jayasinghe, 1999; Reid and Richard, 2004; Viikari *et al.*, 2001**; **Garg *et al.*, 2016**; **Pasha *et al.*, 2013**). Commercial application of pectinolytic enzymes in fruit juice processing can improve the texture, firmness, and clarification of the processed juices (**Fayyaz *et al.*, 1993**).

Fruit juice extraction

Pectinases have potential applications in fruit juice preparation and clarification and have the largest demand among the enzymes those are used in food processing industry. Natural pectins increase the fruit juice viscosity and turbidity. Generally, the consumers prefer less viscous, clear and highly nutritive fruit juice for consumption. Several authors had critically pointed out that pectinases have the ability to decrease the viscosity as well as color; clarification of the fruit juice is also done by enzymatic degradation of pectin polysaccharides of fruit pulps (**Junwei *et al.*, 2000; Kareem and Adebowale, 2007; Chaudhri and Suneetha, 2012; Makky and Yusoff, 2015**). A combine treatment of pectinases, cellulases, arabinases and xylanases can improve the extraction efficiency and yield volume of the fruit juices (**Gailing *et al.*, 2000**). Removal of pectin depends on the biochemical constituents of juice, type of enzyme applied and the duration of incubation (**Versari *et al.*, 1997**). Physicochemical parameters for pectin degradation in juices (orange, dragon, apple, pear, grapes, guava, banana, papaya etc.) have been maintained in following conditions: pH 2.5–6, duration of treatment varies from 5 min to 6 h, temperature ranges below 50°C and the amount of enzyme maintains in ranges from 0.06 to 0.135 % v/w (**Soares *et al.*, 2001; Croaka and Corredig, 2006; Singh and Gupta, 2004; Tochi *et al.*, 2009; Aliaa *et al.*, 2010; Dang *et al.*, 2012**).

Fruit juices contain polysaccharides (pectin, cellulose, hemicellulose, lignin, and starch), protein, tannin, and metals and naturally appear as cloudy (**Vaillant *et al.*, 2001**). The percentage of pectin content varies in fresh fruits (apple - 0.5-1.6, banana - 0.7-1.2, guava - 0.7-1.5, grape - 0.2-1, pineapple - 0.3-0.6, strawberries -

0.6-0.7) which leads to colloid formation and creates problems during the processing of clear fruit juices. **Sulaiman et al., (1998)** reported that suspended particles in the pulp can be eliminated by filtration technique, but the presence of pectin in the solution makes this method difficult. In the food industry, the principal purpose of the clarification process is to eliminate constituents responsible for the turbidity, viscosity and cloudiness in freshly produced juice. Reduction of turbidity by enzymatic depectinisation is also a potential choice for fruit juice preparation (**Kashyap et al., 2001; Landbo et al., 2007**). Generally, different types of pectinases (e.g., pectinase, pectinesterase, polygalacturonase, and pectin lyase) use to degrade pectic substances. Among them, polygalacturonase depolymerises the polygalacturonic acid chain, while pectin lyase depolymerises the high esterified pectin and pectin esterase hydrolyses the methoxy group of the pectin chain. The result is loss of viscosity and cluster formation, which are advantageous in separation through centrifugation or filtration processes. Finally, the juice prepared through this process, appears as higher clarity with more concentrated flavour and colour (**Abdullah et al., 2007; Kaur et al., 2004**). Citrus fruits juices (orange, lemon) are one of the common commercial food products and pectinolyyic enzymes are used to remove the cloudiness and stabilization of juice (**Braddock, 1981**). Application of pectin esterase at the natural condition of citrus fruit juice (pH 3.8) helps to aggregate the cloud particles from the juice within a few minutes **Croaka and Corredig, (2006)**.

Application of pectinases in fruit juice preparation also promotes the liberation of phenolic substances from the fruit skin (**Sharma et al., 2013a**). This treatment increases the phenolics content up to 15 % which indicates the rich antioxidant capacity of the fruit beverages (**Aliaa et al., 2010**). These phenolic components play a significant role to reduce the oxidative stress and exert protective measures against cardiovascular disease and cancers (**Miller and Rice-Evans, 1997**).

Preparation of jams and jellies

During the preparation of jams, jellies, excess amount of sugar is added for gelation. The use of pectin esterase lowers the sugar requirements, because pectin esterase converts high methoxylated pectins into low-methoxylated pectins and ultimately increases gelation property in association with calcium (**Kubra et al., 2018**).

Bio-scouring of cotton fibers in textile processing

Bio-scouring is an ideal eco-friendly technique for removal of non-cellulosic contaminants from the fiber by enzymatic treatment and lowers the toxic effects of chemical detergent like caustic soda. Pectinases along with amylases, lipases, cellulases and hemicellulases have been applied to remove sizing agents from cotton in a safe and eco-friendly manner (**Wang et al., 2007**) which increases the quality of the cotton without any detrimental effect (**Jayani et al., 2005; Klug-Santner et al., 2006**). **Kalantzi et al., (2010)** reported that a combination of lipase and pectinase significantly reduced the treatment time for bio-scouring and produced cotton fabrics with greater quality and excellent dyeing performance.

Degumming/retting of plant bast fibers

Bast fibers are the flexible fibers (skin fiber), contain gum and it supports the cells of phloem, provides strength to the stem. Pectin degrading enzymes are responsible for the retting and removal of gumming agents during the processing of jute, linen from flax, fibers from hemp (*Cannabis sativa*), ramie, kenaf (*Hibiscus cannabinus*), and coir from coconut husks (**Chesson, 1980; Bruhlman et al., 1994**). Retting is a decaying process, in which pectin substances are degraded by certain bacteria (e.g., *Clostridium, Bacillus*) and fungi (e.g., *Aspergillus, Penicillium*) to release the bast fibers associated with phloem tissue beneath the bark (**Sharma and Robinson, 1983**). Removal of gumming agents is the essential step for the use of fiber in textile industry. This degumming process has been made by using pectinases in combination with xylanases; the process is environmentally and economically beneficial practice and lowers the harmful effects of chemical agents used in degumming treatment (**Kapoor et al., 2001**). Fibers are mostly composed by cellulose in associated with hemicellulose (xylan component) and pectin. Pectin degrading microorganisms also secret xylanase but lack of cellulase. Thus, the use of microbial fermentation in jute processing is the advantageous step to increase the quality of jute without damaging the cellulosic fibers (**Gomes et al., 1992**). The microbial process of degumming and retting of fiber crops occur in open fermentation system at alkaline pH (pH 10) by several microorganisms including *Cladosporium, Penicillium, Aspergillus* and *Bacillus* sp. The additionally benefit of fermentation at high pH value lowers the chances of contamination. **Sharma et al., (2012)** reported that the microbial fermentation can able to remove more than 70% ramie gun within 24 h and the quality of processed fibers satisfies the standard of textile industry.

Wine processing

The use of pectinases in the wine production advances the maceration, yield of juice extraction, increases the rate of filtration, and improves the flavour and colour (**Blunt, 2000; Chaudhri and Suneetha, 2012**). Maceration of fruits with pectinases has been done before inoculation of alcoholic fermentation. This process intensifies the quality of wine (**Praveen and Suneetha, 2014**). Pectolytic enzymes are used during wine making from grape mash. This preparation reduces the viscosity of juice. The additional benefit is extraction of anthocyanins due to breakdown of cell structure which acts as color enhancer of the wine (**Tucker and Woods, 1991**). **Bosso (1993)** reported that pretreatment of fermented grapes with pectolytic enzymes gives higher levels of alcohol production. Presence of polymethyl galacturonate esterase (PMGE) in the complex of pectinolytic enzymes increases methanol content as toxic component in wine (**Servili et al., 1992; Revilla and González-SanJosé, 1998**). Methanol is highly toxic for human. It affects central nervous system; the outcomes are decrease level of consciousness, and poor co-ordination. Moreover, vomiting, abdominal cramp, gastrointestinal disorders are the common symptoms. Methanol also gives formate after its metabolism which binds with cytochrome oxidase, starts hypoxia at cellular level and metabolic acidosis. Kidney failure is also possible as long term effects. Therefore, restriction of pectin esterase in commercial mixtures is also essential.

Improvement of chromaticity and stability of red wines

Pectin is the common constituent of plant cell wall of any fruits which lowers the yield of alcoholic fermentation. Application of pectinolytic enzymes for maceration of fruits before alcoholic fermentation by yeast improves chromatic characteristics, turbidity and stability of red wine in comparison with untreated wines (**Revilla and González-SanJosé, 2003**).

Tea and coffee processing

There are mainly three types of tea: black, green and oolong. Green tea is the product of no or little fermentation, oolong tea is semi-fermented and black is full fermented. According to consumption rate, 78% of world's population uses black tea, while the consumption rate of other two types tea are approximately 20% and 2% respectively. The appearance, aroma, taste and color of individual tea are different. These characteristics depend on the oxidation products such as theaflvins, therubigins and other inherent components. Generally, tea leaves contain a wide variety of non-volatile compounds: polyphenols, flavonols and flavonol glycosides, flavones, phenolic acids and depsides, amino acids, chlorophyll and other pigments, carbohydrates, organic acids, caffeine and other alkaloids, minerals, vitamins, and enzymes (Table 4) (**Chaturvedula and Prakash, 2011**). The quality of tea depends on the age of the tea leaves; young tender leaves are the best for production of good quality of tea as compared to old and coarse leaves. The steps of processing of black tea are: plucking → withering → cutting/rolling → fermentation → drying → sorting/cleaning and packing.

Tea leaves content 5-6% pectic substances in the middle lamella of their cell wall; pectinases degrade the pectic substances, accelerates tea processing and reduces the foaming capacity of instant tea powders due to reduction of the water soluble pectin. Application of pectinases enzymes from fungal origin along with cellulase, and xylanase increase the black tea components such as theaflavin (TF 24.77 %), thearubigen (TR 21.52 %), caffeine (CAF), high polymerized substances (HPS 21.54 %), total liquor color (TLC), total soluble solids (TSS 17.49 %) and dry matter content (DMC) (**Murugesan et al., 2002; Marimuthu et al., 2000**). Previously, **Senthilkumar et al., (2000)** reported that crude extract of microbial enzymes from *A. oryzae, A. wentii, A. tamari, A. japonicus, A. awamor*i and *Trichoderma koningii* comprise cellulase, hemicellulase, pectinase, proteinase, and other enzymes; they improve the state of fermentation and also enhance the concentration of TF by 45 %, TR by 48 %, HPS by 33 %, TLC by 19 % and TSS by 3 % respectively. Although, enzyme treatment accelerates tea fermentation at working pH range of 2.5 to 6.0, with optimum pH being between 3.0 to 4.0 and working temperature range of 15 to 50 °C. The concentration of the enzymes have to be controlled carefully to avoid the damage of the tea leaves (**Sharma et al., 2012**), because excess fermentation lowers the glaze-ness and quality of the tea leaves, decreases the color, flavor, and aroma and increases the rate of spoilage of the tea leaves.

Coffee beans are surrounded by mucilaginous (17 % by mass) viscous coat which comprises 84 % moisture with 8.9 % protein, 4 % sugars, 2.8 % pectin and 0.9 % ash (**Murthy and Naidu, 2011**). **Soares et al., (1999)** stated that application of pectinases degrade the outer mucilage cover of the coffee beans and improves the quality of coffee. A previous study had observed that pectinases from *Aspergillus niger* CFR degrades 54-71 % mucilaginous layer within 1 to 2 h of fermentation and the total process had been completed within 3.5 h (**Murthy and Naidu, 2011**). Inoculated waste mucilage of coffee pulp is used as a source of microbial pectinases and recycling process is done by spraying the filtered fermentative liquid over the coffee beans which economized the process (**Soresen et al., 2000; Murthy and Naidu, 2011**).

Table 4 Different components of tea leaves and their beneficial effects.

Class of components	Name of each component	% of dry weight	Contribution
Polyphenols/Flavanols	Total polyphenols	25 – 35	Taste-astringent
	(-) Epigallocatechin gallate	9-14	Give golden-amber color and taste Inhibit histamine release, antiallergic; Act as antimutagenic and carcinogenic; Protects thiol groups of protein. Catechin-potent anti oxidant (25-100 times more than the activity of vitamins C and E).
	(-) Epicatechin gallate	2-4	
	(-) Epigallo catechin	3 – 6	
	(-) Epicatechin	1 – 3	
	(+) Catechin	1 – 2	
	(+) Gallocatechin	1-2	
	Flavonols and flavonol glycosides	3 – 4	Light yellow Acts as anti oxidant
	Polyphenolic acids and depsides	3 – 4	Act as anti oxidant
	Theaflavins	1-2	Taste-astringent Color-yellowish brown
	Thearubigins	15-18	Taste- ashy and slight astringent Color-reddish brown.
Pigments	Pheophorbide		Color- brownish
	Pheophytin		Color- blackish
	Leuco anthocyanins	2 – 3	Color- creamy
	Carotene		Color- yellow
	Chlorophylls and other pigments	0.5 – 0.6	Color and appearance
Alkaloid	Caffeine	3 – 5	Taste- bitter
	Theobromine	0.2	Stimulates cardiac activity, dilates vessels of heart, brain and bronchi, has antispasmodic and diuretic effects.
	Theophylline	0.5	Potent diuretic agent and also acts as vasodilator agent in heart, brain and bronchi, stimulates CNS.
Acids	Amino acids	2-4	Taste- brothy Produces volatile carbonyl compounds during processing.
	Organic acids Citric, tartaric, malic, oxalic, fumaric and succinic acids, palmitinic acid)	0.5 – 2.0	Stimulate release of gastric juice, regulate the state of intestinal flora, support normal acid-base balance, activate peristaltic movements and decrease risk gastro-intestinal diseases.
carbohydrates	Monosaccharides	4 – 5	
	Polysaccharides	14 – 22	
	Cellulose and Hemicellulose	6-8	
	Pectins	5 – 6	Cell wall component
	Lignin	5 – 6	
General components	Protein	14 – 17	
	Lipids	3 – 5	Have significant role in formation of aroma.
	Ash (minerals)	5 – 6	
Aroma generating compound	Volatiles (more than 600 types)	0.01 – 0.02	Aroma
Flavouring agents	Linalool, linalool oxide		Flavour- sweet
	Geraniol, phenylacetaldehyde		Flavour- floral
	Nerolidol, benzaldehyde, methyl salicylate, phenyl ethanol		Flavour- fruity
	Trans-2-hexenal, n-hexanal, cis-3-Hexenol, grassy, β-ionone		Flavour- fresh flavour

Waste water treatment

Pectinaceous materials are commonly present in the wastewater of citrus fruits processing industry. Initially physical and chemical treatments (physical dewatering, chemical coagulation, chemical hydrolysis and methnogenesis) were the general practice for waste treatment to remove the pectinaceous materials. This process is not cost effective, time saving and eco-friendly, and creates chemical hazards. Pretreatment by pectinolytic microbes (alkalophilic *Bacillus* sp. GIR 621) during the activated-sludge treatment decomposes the pectinaceous materials from waste water (**Tanabe *et al*., 1986; Tanabe *et al*., 1987**). Food industry discharge waste effluents during processing of vegetables which carries pectin as by-product. Pectinases are used for the pretreatment of the wastewaters to remove pectin from it (**Jayani *et al*., 2005**). The application of pectinolytic organisms during activated-sludge treatment is an eco-friendly, cost effective, timesaving process.

Paper and pulp industry

In the modern biotechnological era, paper and pulp industries have started to exploit enzymes like xylanases, ligninases, mannanase and pectinases for biobleaching (**Kirk** and **Jefferies, 1996; Bajpai, 1999; Kubra *et al*., 2018**). Formation of sheet is a vital step in papermaking and the presence of pectins in the pulp weakens dewatering due to their high cationic demand, causes yellowness of paper (**Garg *et al*., 2016**). This problem can be solved by using pectinase (biobleaching agent) which starts depolymerization of pectin, and consequently lowers the cationic demand of pectin as compared to peroxide bleaching (**Reid and Ricard, 2000; Viikari *et al*., 2001**). The application of pectin degrading enzymes in paper manufacturing reduces the use of cationic polymers. Alkaline pectinase from *streptomyces sp*. was employed in bio-bleaching of pulp. Alkaline pectinases used in Japanese paper manufacturing system. This treatment improves the strength of the pulp as compared to the

conventional soda-ash cooking method (**Kubra *et al*., 2018**). The bio-bleaching treatment of hardwood and bamboo kraft pulps is mediated by microbial pectinases and other polysaccharidases. This eco-friendly process maintains the quality and brightness of the paper as similar as conventional method but lowers the release of eco-toxic agents like organochlorine compounds in the waste water (**Ahlawat *et al*., 2007, 2008; Dhiman *et al*., 2009; Kaur *et al*., 2010**).

Animal feed Processing

Pectinolytic enzymes are applied for production of animal feeds, as the enzymes hydrolyze non-degradable bio-fibers and change the palatability of feed. The application of pectinases in animal feed production improves the quality of feed by lowering the viscosity and increases the absorptivity of nutrients. Moreover, the enzymatic treatment reduces the fiber content of the feed and enhances the liberation of nutrients trapped under the non-degradable fibers. Low fiber containing diet also reduces the amount of feces which is ecologically beneficial (**Jayani *et al*., 2005; Praveen and Suneetha, 2014**). The use of multiple enzymes like xylanases, pectinases and cellulases during preparation of ruminants' diet economically improve the quality of feeds. This supplementation increases the digestibility of organic matter by changing the fiber digestion in rumen and increases the dietary energy uptake capacity (**Selinger *et al*., 1996; Petersen, 200l; Arambel *et al*., 1987; Ghorai *et al*., 2009**). All these factors ultimately help to improve the animal weight.

Purification of plant viruses

A pure preparation of plant viruses is essential to study the physico-chemical and biological characteristics of them. In certain condition virus particles are associated to phloem tissue; application of alkaline pectinases and cellulose degrade the fibers and remove the virus form the tissue in a pure form (**Jayani *et al*., 2005**).

Vegetable oil extraction

Traditionally, organic solvent (hexane) was used for extraction of edible oil from oil producing crops like rape seed, coconut germ, sunflower seed, palm and olives. Organic solvent like hexane is a potential carcinogen and increase the risk cancer (**Kashyap *et al*., 2001**). Bio-extraction of vegetable oils in aqueous phase can be possible by using alkaline pectolytic enzymes which rupture the plant cell wall after degradation of pectin in middle lamellae and promote the extraction of oils from the inner part of the seeds or germ layer. Application of a mixture of cellulases, hemicellulases and pectinases in oil extraction process increases the maximum yield of oil uptake. This process also improves the polyphenolic and vitamin E content within the oil and enriches the quantity of antioxidants (**Kashyap *et al*., 2001; Hoondal *et al*., 2002; Iconomou *et al*., 2010**).

Saccharification of agricultural substrates

Saccharification of agro-industrial wastes in presence of pectinases increases fermentable sugar content in the mixture which is used for bioethanol production (**Biz *et al*., 2014**). Different polysaccridases (pectinase, hemicellulases and cellulases) are being used for saccharification of plant cell wall polysaccharides including pectic substances into simple sugars which are used in bioethanol production (**Beldman *et al*., 1984; Hossain *et al*., 2011; Collares *et al*., 2012**).

Recycling of wastepaper

Chemically deinking process creates environmental hazards; but, enzymatic deinking reduces pollution hazards, energy consumption, disposal problems and improves the performance. A cluster of enzymes (pectinases, hemicellulases, cellulases and lignolytic enzymes) are applied during deinking process. Basically, enzymes modify the bonding characteristics between ink and fiber; the result is removal of ink from the surface of the fibers during washing (**Xu *et al*., 2009; Pathak *et al*., 2010; Xu *et al*., 2011**).

A COMPARATIVE OVERVIEW ON THE MAJOR POLYSACCHARIDES DEGRADING ENZYMES

Table 5 Comperative features among the pectinases, amylases, cellulose and xylanases.

Enzyme Features	Pectinases	Amylases	Cellulase	Xylanases
Types	Hydrolases: Pectin esterase, Exo and Endo polygalacturonase, Exo and Endo polymethylgalacturonases, Rhamnogalacturonases, Xylogalacturonase. Lyases: Exo and Endo polygalacturonate lyase, Exo and Endo polymethylgalacturonate lyase, Rhamnogalacturonan lyases.	Hydrolases: α- amylase, α-glucosidase, Isoamylase, Pullulanase, β-Amylase, γ-Amylase Transferases: Branching enzyme, Cyclodextrin glycosyl transferase.	Endo-(1,4)-*β*-D-glucanase (Endoglucanase), Exo-(1,4)-*β*-D-glucanase or 1,4-β-D-glucan-cellobiohydrolase (Exoglucanase), β-Glucosidase.	Endo-1,4-β-xylanase, β-D-xylosidase (xylan-1,4-β-xylosidase), Acetylxylan esterase, α-glucuronidase, α-arabinofuranosidase.
Structure	Single domain consists of right-handed parallel β-helix composed by parallel β-strands, the central cylinder contains seven to nine complete helical turns Active site - Lyase: Asp, Arg Polygalacturonase: Asp, His	α-amylase family: primarily has three domains (A, B, C) A-domain, consists of (α/β)$_8$ or TIM barrel structure. Extra domain may also be present. β-amylase has single domain with (α/β)$_8$ barrel core Active site – α-amylase and others: Asp, Glu and Asp β-Amylase: Cys, Ser.	Most cellulases consists of multiple domains which are joined by linker sequences, contain Ser, Thr and Pro. Cellulases also possess (α/β)$_8$ or TIM barrel structure. The catalytic centre is located at the C-terminal ends of the beta strands; aromatic residues forming the substrate binding site. Bacterial cellulase system is more complex, forms, cellulosomes.	Xylanases consists of multiple domains, joined by linker sequences. Enzymes possess (α/β)$_8$ or TIM barrel structure and also have β-jelly roll fold structure.
Mechanism of catalysis	Hydrolases – Endo-type: Random cleavage Exo-type: Terminal cleavage Lyases: Endo-type: Transelimination, random cleavage Exo-type: Transelimination, terminal cleavage.	Hydrolases – Endo-acting: α- amylase - α-retaining double displacement mechanism; acid base catalysis; Glu acts as proton donor and one Asp acts as nucleophile.	Acid base catalysis; two glutamates act as the catalytic residues (one Glu at β-strand IV is proton donor, another Glu at β-strand VII is nucleophile.	Acid base catalysis (double displacement mechanism); Two glutamates act as proton donor and nucleophile at the catalytic site.
End products	Esterases: Pectic acid + methanol / ethanol. Endo-type hydrolases: Oligo-galacturonates or its methyl derivative.	Endo-acting: Several maltooligomers, branched dextrins , maltose. Exo-acting: maltose, and glucose.	Endoglucanase-Reducing or non-reducing cellooligosaccharides; Exoglucanase- Cellobiose β-Glucosidase-	Xylanase produces xylooligosaccharides. β-xylosidase releases xylose residues from the nonreducing ends of

	Exo-type hydrolases: Mono / di-galacturonate or its methyl derivative. .Endo-type lyases: Unsaturated oligo-galacturonates. Exo-type lyases: Unsaturated Mono / di-galacturonates.		Cellobiose to glucose	xylooligosaccharides. Acetylxylan esterase removes acetyl group.
Assay methods	Esterase: Estimation of methanol; Hydrolases and lyases: Colorimetric assay for estimation of reducing sugar by 3,5 dinitrosalicylic acid reagent; Chromatographic study for methyl derivatives of galacturonate.	Estimation of reducing sugar by 3,5 dinitrosalicylic acid reagent. Determination of blue loss property of starch iodine color complex.	Estimation of reducing sugar by 3,5 dinitrosalicylic acid reagent. β-glucosidase activity- Spectrophotometric determination of p-nitrophenol from the substrate p-nitrophenyl-β-D-glucoside.	Estimation of reducing sugar by 3,5 dinitrosalicylic acid reagent or by using copper sulfate and areno-molybdic acid reagent.
Applications	Fruit juice preparation, degumming, tea and coffee processing, bio-bleaching in paper industry, vegetable oil extraction, animal feed processing, recycling of waste paper, waste water treatment.	Liquefaction of starch for production of glucose syrup, maltose, high fructose containing syrup, maltooligosaccharides and others. Used in backing and brewing industry. Textile industry for desizing. Clinical use- medicine as digestive aids. Production of ethanol. Used in detergents and waste water treatment.	Pulp and paper industry, textile industry, bioethanol production, wine and brewing industry, clarification of fruit juices, olive oil extraction, carotenoid extraction, animal feed production, detergents industry, waste management.	Pulp or paper industry, food industry, extraction of coffee and plant oils, clarification of fruit juices and wine, animal feed processing, textile industry, biofuel production.

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