

Biological Function(s) and Application (s) of Pectin and Pectin Degrading Enzymes

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Pectin is an integral part of plant cell wall and since centuries pectin extracted from plants is widely used in food and fruit juice processing. Moreover, in last half century, the applications have also invaded into many bio-processing applications such as pharmaceutical, bioenergy, textile, paper and tea processing. In these growing industries, the use of pectinases has grown with a significant amount i.e. approximately 10 % of total global enzyme market comes from pectinases. Herein comprehensive analyses of information related to structure and function of pectin in plant cell wall as well as structural classes of pectins have been discussed. The major function of pectin is in cementing the cellulose and hemicelluloses network, cell-cell adhesion and plant defence. Keeping the wide use of pectin in food industry and growing need of environment friendly technology for pectin extraction has accelerated the demand of pectin degrading enzymes (PDEs). PDEs are from three enzyme classes: carbohydrate esterases from CE8 and CE12 family, glycoside hydrolases from GH28 family and lyases from PL1, 2, 3, 9 and 10. We have reviewed the literature related to abundance and structure-function of these above mentioned enzymes from bacteria. From the current available literature, we found very limited information is present about thermostable PDEs. Hence, in future it could be a topic of study to gain the insight about structure-function of enzymes together with the expanded role of thermostable enzymes in development of bioprocesses based on these enzymes.

Keywords: Pectin, Pectin degrading enzymes (PDEs), Glycoside hydrolases, Pectate lyases.

Pectin is abundant in middle lamella and primary cell wall of various terrestrial plants and tissue types like fruits and leaves (Rose 2003; Lerouxel *et al.* 2006; Scheller *et al.* 2007). The amount of total pectin is highest in dicotyledonous plants and nongraminaceous (nongrass) monocots such as apples, oranges, cherries, sunflowers, grapes, bananas and tomatoes. It is also present in grasseous plants like maize, wheat, sorghum,

barley etc but total amount is lower than dicots. Primary cell wall of dicot plants contain 35% pectin while grasses have approximately 2-10% pectin (Cosgrove 2005; Wolf and Greiner 2012; Atmodjo *et al.* 2013). Pectins extracted from orange and apple is currently major source and in extraction role of pectin degrading enzymes are central. In this review, current status of literature and future trends has been described in detail.

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Functional Role of Pectin in plant cell wall

As a part of the cell wall, pectin plays major role in providing structural support to plant (Palin and Geitmann 2012; Wolf and Greiner 2012; Atmodjo *et al.* 2013; Daher and Braybrook 2015). Primary cell wall is very rich in a cross-linked network of cellulose and hemicelluloses but role of pectin is pivotal in cementing this network. The structural studies present a model in which the role of pectin is modelled as a matrix into which the cellulose and hemicelluloses fibrils are embedded and glued together as a structural unit. A representative depiction of a typical primary cell wall is shown in figure 1. From the proposed model, the special role of pectin is very significant and mainly lies in its intrinsic capability to form a coagulated gel. Indeed, the name pectin is derived from the greek word $\rho\epsilon\kappa\tau\omicron\varsigma$ that stand for congealed or coagulated. The middle lamella, as shown in figure 1, contain abundant of pectins and these pectins play a necessary role in the formation of cell-cell adhesion among various plant tissues (Willats *et al.* 2001; Malinovsky *et al.* 2014). Here, the role of calcium in formation of adhesive interaction is well documented and experimentally confirmed too. Calcium cross-links the pectin component of two middle lamellas through the negative charge present in the monomeric unit galacturonic acid. Other factors responsible for cell-cell adhesion and interaction are the side chain sugars attached to backbone formed by polygalacturonan (Scheller *et al.* 2007; Palin and Geitmann 2012). Among these side-chains, some sugars some are very common hexose and pentose sugars such as arabinose, galactose and xylose but many of them are peculiar and rare sugars like apiose and aceric acid- a branched chain sugar, 3-deoxy-manno-2-octulosonic acid (KDO) - an eight carbon containing sugar and 3-deoxy-lyxo-2-heptulosaric acid (DHA)-a seven carbon containing sugar. In literature, several plant mutants lacking these rare sugars have been reported and in these mutants loss of cell-cell adhesion have been noticed because of the altered composition of the pectin in middle lamella (Cosgrove 2005; Mohnen 2008; Daher and Braybrook 2015). In addition, pectin has also an effect on porosity, surface charge, pH and ion balance in the cell wall.

Furthermore, pectin oligosaccharides are known to activate the plant defence mechanism

by accumulation of phytoalexin, which displays antimicrobial activity and also stimulate the lignification in plant tissues (Malinovsky *et al.* 2014). Phytopathogenic fungi secrete pectin degrading enzymes (PDEs) to gain the entry into the plant tissues. Subsequently, PDEs produce oligogalacturonide (OGA) fragments from the pectin backbone, which act as potent defence response elicitors (Collmer and Bateman 1982; Gonzalez-Candelas and Kolattukudy 1992; Lara-Márquez *et al.* 2011; Xie *et al.* 2012; Tsuyumu *et al.* 2014).

Altogether, pectin is mainly a structural polysaccharide present in the plant cell wall and has major function in holding the overall structure of the plant together with cellulose and hemicelluloses network (Lerouxel *et al.* 2006; Malinovsky *et al.* 2014; Tsuyumu *et al.* 2014). Moreover, it also plays a significant role in plant-pathogen interaction, where degradation products of pectin have been shown as a major molecule to activate the pathogen protection machinery present in plants.

The Structural classes of Pectin

Pectin is a hetero-polymer and perhaps the most complex macromolecule present in the plant cell wall, having 17 different types of monosaccharide as well as 20 different types of linkages to assemble these sugars (Rose 2003; Atmodjo *et al.* 2013). Nevertheless, approximately 65% of cell wall pectin is a homopolymer of α -D-galacturonic acid (figure 2). Hence, α -D-galacturonic acid is the major monosaccharide found in pectin. However, studies of sugar compositions and its linkages have led to the view that there are three major type of pectic polysaccharides are present: (1) Homogalacturonan (HG), (2) Rhamnogalacturonan I (RG-I) and (3) Rhamnogalacturonan II (RG-II) (Mohnen 2008). In figure 2, a simplified representation of all three groups of pectin is shown (Scheller *et al.* 2007; Harholt *et al.* 2010).

Homogalacturonan (HG)

HG is a homopolymer of α -D-galacturonic acid. The glycosidic linkage between the backbone is a α -1, 4 configuration. The monomeric unit is a sugar acid, an oxidized form of D-galactose. A pyranose ring form of the α -D-galacturonic acid is shown in figure 2. Based on this, it has an aldehyde group at C1 and a carboxylic acid group at C6. The carboxylic acid at position C6 occurs in the form

of methyl ester too (As shown in figure 2). Degree of esterification (DE) determines the physical properties of the pectin and based on esterification, industrial quality of pectins are classified into two broad categories: HM (high methoxy) and LM (low methoxy) pectin as described by IPPA-international pectin producers association). HM pectin has greater than 50% DE whereas LM pectin has less than 50% DE. HM and LM pectins form the gel by different mechanism because of differential amount of esterification (Leroux *et al.* 2003; Caffall and Mohnen 2009). HM pectin requires a minimum amount of soluble solids such as sugars and a narrow pH range, around 3.0, in order to form gels. Also, these gels are thermally reversible. In general, HM-pectins are hot water soluble and often contain a dispersion agent such as dextrose to prevent lumping. In comparison, LM-pectins yield gels independent of sugar content and the gel formation is not sensitive to pH. LM-pectins need presence of a controlled amount of calcium or other divalent cations for gelation. Based on these properties they have their unique set of industrial applications (May 1990; Dominiak *et al.* 2014b). Like, esterification, also in the some cases the amidation of carboxyl group at C6 position occurs but the amount of amidation is too low to influence the gelling properties (Scheller *et al.* 2007; Caffall and Mohnen 2009). Similarly acetylation of the oxygen atom of position O2 and O3 has been also seen in some natural pectin. Acetylation of α -D-galacturonic acid changes the distinct gelling properties of pectin in negative manner.

Rhamnogalacturonan II (RG-II)

RG-II amount to approximately 10% of the total pectin and is the most composite but structurally the most conserved group of pectin found in nature. It has similar backbone like HG but there are four conserved side chains A, B, C and D are invariably present. Side chain A and B are an octasaccharide and nonasaccharides respectively linked to HG backbone via α -D-Apiosyl (Apif) at O2 position. Side chain C and D are chemically a disaccharide and is linked to O3 position of polygalacturonate backbone. RG-II has 12 different types of sugars in more than 20 linkages. The conserved nature of RG-II pectins suggests a key function in plant growth and development (Srivastava and Malviya 2011; Atmodjo *et al.* 2013). Expectedly, it has been reported that a

minor modification of these conserved sugar and linkage pattern may result into a deleterious effects on plant growth including dwarfism. Also, in the network structure of the cell wall, generally, they are cross-linked via borate in the plant tissue (Palin and Geitmann 2012; Atmodjo *et al.* 2013).

Rhamnogalacturonan I (RG-I)

It comprises 20-35% of total pectin and is significantly unrelated in the backbone structure. HG and RG-II has a similar backbone of α -1,4 linked D-galacturonic acid but here the backbone is a repeating unit of a disaccharide: $-[4)\text{-}\alpha\text{-D-galacturonic acid-(1,2)\text{-}\alpha\text{-L-rhamnose-(1)}$. Also, in HG the most abundant modification is esterification at C6 position whereas in RG-I, the acetylation at O2 and O3 position of α -D-galacturonic acid is more prevalent (Scheller *et al.* 2007; Caffall and Mohnen 2009).

Very clearly, the HG is most abundant pectin but on the whole, in a typical plant cell wall, how these three types of pectin are arranged and cross-linked to each other is not well understood (Willats *et al.* 2001; Mohnen 2008; Atmodjo *et al.* 2013). Nonetheless, the experimental reports are indicative of covalent linkages between them. One of the roadblocks to gain further understanding about the structural association of these three type of pectin lies in the isolation of intact native pectin from the plant cell wall. Generally, chemicals and enzymes used in isolation and enrichment of pectin fraction destroy the structural linkages present in the native pectin (Mohnen 2008; Caffall and Mohnen 2009). Therefore, in future, more advanced techniques of cell imaging will be indispensable to gain insight into function of diverse classes of pectin in cell wall.

Applications of Extracted Pectins

Pectin is common in daily used cereals and fruits so, the intake of pectin in human diet is obvious and in the digestive system it is metabolized by the gut-microbes. Hence, intake of pectin in human diet has prebiotic effect as well as it is an excellent source of soluble dietary fibre (Thakur *et al.* 1997; Sriamornsak 2003; Munarin *et al.* 2012; Garg *et al.* 2016). There are several reports linked to consumption of pectin as a food material. One of the study shows that the chamomile extract from vegetable pectin reduce the diarrhoea related symptoms (Iglesias and Lozano 2004). Another study revealed that the

pectic oligosaccharides found in carrot soup are very effective in minimizing the gastro-intestinal infections (Srivastava and Malviya 2011; Munarin *et al.* 2012). Furthermore, the pectin intake lowers the blood cholesterol level and act as controlling agent of blood glucose level in diabetic patients (Srivastava and Malviya 2011; Munarin *et al.* 2012). In addition, from decades pectin is used in food products like Jam, yoghurt drinks, jelly and fruity milk drinks, predominantly as gelling, stabilizing or thickening agent (May 1990; Sriamornsak 2003; Sharma *et al.* 2006; Xiao and Anderson 2013; Latarullo *et al.* 2016). According to IPPA (International Pectin Producers Associations), pectins have following major applications: (1). Fruit applications: Jams, jellies, and desserts ;(2). Fruit preparations used in bakery fillings and toppings ;(3) Dairy industries: acidified drinks and protein drinks ;(4) Confectionery and beverages; (5) Nutritional and health products and (6) Pharmaceutical and medical applications (BeMiller 1986; Thakur *et al.* 1997; Sriamornsak 2003; Sharma *et al.* 2006; Munarin *et al.* 2012; Tapre and Jain 2014; Garg *et al.* 2016).

Although structurally, pectins are of three major class but applications are more confined to homogalacturonan. Also, pectins in these abovementioned applications are extracted from plant sources, so US Food and Drug administration (FDA) has categorised them as GRAS (generally regarded as safe). As per recommended specifications for food grade pectin, the total galacturonic acid should be more than 65% calculated on the ash-free and dried bases. In some cases, the food grade pectin has amidation at C6 position and as per US FDA; the amount of amidation should not exceed 25% of total carboxyl groups. The data is freely available at the website: <https://www.fda.gov/food/ingredientpackaginglabeling/gras/scogs/ucm260947.html> and <http://www.ippa.info/safety.html>.

At present, the most common source of pectin is citrus and apple peels. The literature available on extraction methods are of two types: (1) direct boiling at low pH and (2) microwave based heating (Canteri-Schemin *et al.* 2005; Xu *et al.* 2014). Boiling is a conventional method and takes more than 2-4 hours to obtain the sufficient amount of pectin, later which is precipitated by organic

solvents like methanol or ethanol. Although, it is a traditional method but there are few drawbacks like long duration and thermal degradation of the pectic substances. On the contrary, the microwave heating procedure does not take more than 15-20 minutes and produces the good quality pectin with a satisfactory yield (Canteri-Schemin *et al.* 2005; Liu *et al.* 2006; Ismail *et al.* 2012; Methacanon *et al.* 2014; Xu *et al.* 2014; Lefsih *et al.* 2017). Here, the both methods employed for extraction of pectin is based on use of nitric, hydrochloric or sulphuric acid. Therefore, the main disadvantage of this technology is generation of large volumes of acidic effluent, which need additional treatment before release into environment. Hence, enzymatic degradation to release the pectin from plant cell wall and thereby achieve sustainable, green production of pectin has become a topic of research in the last decade (Dominiak *et al.* 2014a; Wikiera *et al.* 2015). Consequently, now these days enzymatic processing of natural plant based material to obtain a sizeable amount of intact and functional pectin is more favoured.

Enzyme classes for Pectin Degrading Enzymes (PDEs)

Although there are three types of pectin present in plants but the major industrial and biotechnological applications are restricted to HG type (BeMiller 1986; Sharma *et al.* 2006). Thus, in this section for classification of PDEs, only those enzymes which are capable for hydrolyzing HG are included. For a better understanding the distinction between pectin and pectate lies in esterification. The former has esterification at C6 position whereas latter completely lack esterification. As discussed earlier based on extent of esterification there are two grades of pectin – low and high methoxy pectin.

According to type of linkages there are two categories of enzymes: de-esterifying and de-polymerising enzymes. The later enzymes are further classified into two classes based on the reaction mechanism – hydrolases and lyases (Dubey *et al.* 2010; Hugouvieux-Cotte-Pattat *et al.* 2014). Both enzymes break the α -1,4 linkages present in polygalacturonate but the former one use water for hydrolysis while the later one cleave the linkage, without water molecules, via β -elimination reaction (Tapre and Jain 2014; Garg *et al.* 2016; Yadav 2016). A simplified pictorial representation

of the reaction, reaction products and position of bond cleavage is shown in figure 3 and enzyme classes are listed in table 1.1. Based on these, the pectin degrading enzymes (PDEs) are described below.

Pectin methylesterase (PME) (EC 3.1.1.11)

Perform the removal of the methoxyl groups from the C-6 carboxyl groups of galacturonate units by hydrolysis (Fig. 3). Subsequently, pectin acid, methanol and H⁺ from ionisation of carboxyl groups are formed. The release of H⁺ decrease the pH of the reaction media so, by the measurement of the pH of the solution after the reaction is an assay to determine the enzymatic activity (Yadav 2016).

There are many PME secreting microbes and these enzymes are listed and classified in a comprehensive database - CAZy (Carbohydrate Active enZymes) (Cantarel *et al.* 2009). In this CAZy database, PME belongs to carbohydrate esterase (CE) family and this group of enzyme is active on carbohydrate esters. Currently, there are seventeen classes (CE1 to CE16 and one unclassified family) of CEs and PMEs are grouped into CE8 family. This classification is based on evolutionary history of the genes encoding the PMEs and also it takes account of sequence similarities among the genes, reaction mechanisms and protein folds. So far, total 2794 gene sequences are reported in the CE8 family and a greater part of them are from bacteria and eukaryotes. From archaea, only 5 genes are reported till now. Among these only 47 proteins are studied in detail and from these, 8 proteins have three-dimensional structures deposited in Protein Data bank (PDB) (Laskowski 2001).

Here, our focus is on bacterial pectin degrading enzymes (PDEs) and among the 47 biochemical characterized enzymes; only three enzymes have been reported till date (as per the information available in public database) from bacterial domain (Marín-Rodríguez *et al.* 2002; Dubey *et al.* 2010; Hugouvieux-Cotte-Pattat *et al.* 2014) Interestingly, all the three enzymes are from family *Enterobacteriaceae*. These three enzymes are: (1) pectin methyl esterase (PmeA) of *Dickeya chrysanthemi* B374/B364 (NCPPB898); (2) pectin methyl esterase A (PemA) of *Dickeya dadantii* 3937 and (3) pectin methyl esterase B (PemB) of *Dickeya dadantii* 3937 (Cantarel *et al.* 2009)

The recombinant purified PmeA displayed the maximum activity at pH 8.0 – 9.0 and at a temperature of 50°C. For the optimal activity, 50-100 mM monovalent cations or 5-10 mM divalent cations are essential and a specific effect of Ca²⁺ and Zn²⁺ on PmeA activity has been also seen (Plastow 1988; Shevchik and Hugouvieux-Cotte-Pattat 1997). The other two enzymes – PemA and PemB has also similar enzyme profile. Currently, available commercial preparation of PROZOMIX is from *Streptomyces avermitilis MA-4680*, but data related to biochemical studies is not available. From the specification sheet of the enzyme from the supplier, the optimum activity is at 30°C and pH 7.5. The three dimensional structure of PmeA and PemA confirms the presence of a typical α -helix found in PMEs (Plastow 1988).

Pectin acetylerase (PAE) (EC 3.1.1.06)

Hydrolyze the pectin by removing acetyl groups from C2 and C3 hydroxyl groups of galacturonate units present in HG and RG-I and RG-II. The first pectin acetylerase activity was reported in a fungus - *Aspergillus niger* (Kauppinen *et al.* 1995; Dinu *et al.* 2007; Martens-Uzunova and Schaap 2009). However, the studies of pectin acetylerase are so far not enough to get a molecular detail of the enzyme activity and substrate specificity. PAEs cleave the acetyl ester linkage in the homogalacturonan region of pectin yielding pectic acid and acetate. Likewise PMEs, PAEs are classified into carbohydrate esterase (CE) families 12 and 13. There is a very little information about the members of CE13 family so; the knowledge of function and substrate specificity is from members of CE12 family.

At this time, there are 1216 gene sequences are available and more than 80% of these sequences are from bacteria and among the bacterial PAEs, only eight have been studied in terms of structures and functions. Pectin acetylerase (*YesY;BsPAE*) of *Bacillus subtilis* and pectin acetylerase (*PaeY;Dda3937_03373*) from *Dickeya dadantii* 3937 are the most studied PAEs and have the optimum pH and temperature in the range of 7.0-9.0 and 40-50°C (Shevchik and Hugouvieux-Cotte-Pattat 2003; Grenier *et al.* 2006). The enzyme profile data is from the synthetic substrate- pNP-acetate so; still the enzymatic activity with native substrate acetylated pectin has been not established. Surprisingly, there is no

report of the confirmed three dimensional structure by X-ray crystallography or NMR (Nuclear Magnetic Resonance) for any characterized protein, yet the evolutionary history predicts a unique structure of (α / β)-sandwich unlike the α -helix of PMEs. In addition, CE12 family has also rhamnogalacturonan acetylsterases (RGAE) that carry out hydrolytic cleavage of acetyl groups from rhamnogalacturonan chain of RG-II. Commercially supplier PROZOMIX has PAE from *Clostridium thermocellum* which has the optimum temperature at 50°C and a pH of 6.5 and to the best of our knowledge there are no supplier of RGAE (Searle van Leeuwen *et al.* 1992; Remoroza *et al.* 2014).

Conclusively, de-esterifying enzymes aim to remove the modifications like addition of methoxy and acetyl group. Although, we have not discussed the removal of sugar modifications of the polygalacturonate backbone but we need a set of specific hydrolases targeting the side chain sugars, as described earlier in the section “structural elements of pectin”. These specific and unique enzymes have been reviewed elsewhere in details (Atmodjo *et al.* 2013; Ndeh *et al.* 2017).

De-polymerising enzymes (Hydrolases)- Endopolygalacturonase (EC 3.2.1.15) and Exopolygalacturonase (EC 3.2.1.67)

These are known as polygalacturonases (PG) and catalyze the hydrolysis of α -1,4-glycosidic linkages in polygalacturonic acid (PGA). Depending on the site of cleavage they are further divided as Endo-PG that cleaves the glycosidic bonds randomly and yields oligogalacturonate and Exo-PG that cleaves the bond from terminal and generally produces monogalacturonates. In addition, there is another class of Exo-PG, known as exo-poly- α -galacturonosidase (EC 3.2.1.82), that cleaves the penultimate bond rather than terminal bond from non-reducing end of PGA. Consequently, they produce digalacturonate as a product rather than monogalacturonate.

All three types of hydrolases are clustered into glycosyl hydrolase 28 family (GH28). So far, 21 bacterial proteins have been characterized as a member of GH28 and of these 12 are endo-PG, 7 are exo-PG and remaining 2 are exo-poly- α -galacturonosidase. Among these, there are four enzymes with their three-dimensional structures deposited in PDB. Structural information of these four proteins reveals a α -helix fold like PME. The

overall fold is right-handed parallel α -helix, which is decorated with loops and secondary structure elements on one side (“top”), and a 26-residue N-terminal extension comprising of α -helix and a loop at the “bottom”. At the top side there is a conserved active site pocket, in which three aspartate residues are present at the bottom of the pocket. These aspartate residues are responsible for the hydrolysis of the glycosidic linkages present in the pectin and pectate and are known as active site residues. Based on the mechanism, these are inverting glycoside hydrolases where upon hydrolysis the anomeric configuration inverts in product. All three enzymes produce reducing sugars as a reaction product so, the enzyme assays to measure the Endo and Exo-PG activity is the standard assay for estimation of reducing sugars i.e 3,5-dinitrosalicylic acid (DNS) assay described by Miller (Miller 1959).

De-polymerising enzymes (Lyases)- Endopolygalacturonate lyase (EC 4.2.2.2) and Exopolygalacturonate lyase (EC 4.2.2.9):

The enzymes from EC 4.2.2.2/9 is an example of carbon-oxygen lyase. Besides, lyases have also the ability to break the carbon-carbon, carbon-nitrogen, carbon-sulfur, and carbon-halide and phosphorus-oxygen bond present in organic and inorganic compounds. The substrate utilized by pectate lyases (PL) is same like endo and exo-PG and cleaves the same carbon-oxygen bond of α -1,4 glycosidic linkage between uronic acids but instead of yielding the saturated mono, di and oligogalacturonic acids, PL produces the same product having a unsaturated C4-C5 bond. Also, these unsaturated oligo-galacturonates absorbs at 235nm, which is utilized for *in vitro* assay of pectate lyases. Thus far, an alkaline pH range 8.5-10.5 and need of a metal ion is the general trend exhibited by most of pectate lyases. Ca^{2+} , Co^{2+} , Mn^{2+} , and Ni^{2+} are mostly used as a metal cofactor in the catalysis (Yadav 2016).

Like PGs, pectate lyases are also further divided as endo-PL that cleaves the glycosidic bonds randomly and exo-PL that cleaves the bond from terminal and generally produces unsaturated monogalacturonates. In addition, there is another class of exo-PG, known Oligo-D-galactosiduronate lyase (EC 4.2.2.6), that cleaves the penultimate bond rather than terminal bond from non-reducing end of PGA and yields di-galacturonates. In CAZY

database, these enzymes are found in five different polysaccharide families: PL1, PL2, PL3, PL9 and PL10. Except PL1, which has more than 100 PLs from bacterial sources and these are well studied, remaining four pectate lyases have only a few well studied PLs from bacteria. Three-dimensional structures of many PLs have been determined and three different kinds of topologies are present in the five different families for the catalytic module: (1) a right-handed α -helix fold which is common with the families PL-1, PL-3, and PL-9; (2) an (α/α) 7 toroid in family PL2; and (3) (α/α) 3 toroid in family PL-10. α -helix fold is more abundant among different pectin degrading enzymes and is structurally very unique. As mentioned earlier, PME and PGs have also this typical α -helix fold (Yoder *et al.* 1993; Hurlbert and Preston 2000).

Despite variations in sequence similarities and protein folds of all five families, they still have a shared catalytic mechanism. On the contrary to glycoside hydrolase (PGs), polysaccharide lyase (PLs) breaks bond without involvement of water molecule and result in the formation of an unsaturated bond at the non-reducing end. The catalytic mechanism can be summarized into three steps: (1) abstraction of the C-5 proton on the sugar ring of a galacturonic acid or ester by a basic amino acid from the active site, (2) stabilization of the resulting anion by charge delocalization into the C-6 carbonyl group and (3) lytic cleavage of the O-4: C-4 bond, facilitated by proton donation

from a acid residue from the active site to yield a hexenuronic acid and hexuronic acid (Garg *et al.* 2016; Praveen and Suneetha 2016).

Applications of Pectin Degrading Enzymes (PDEs)

In the previous section, we described the salient features of the enzymes linked to pectin degradation and now in this section, the applications of PDEs are outlined. The first commercial application of PDEs was for clarification of apple juice in 1930 by Kertesz. Since then, it has expanded for many applications as outlined in figure 4.

Fruits and vegetables processing

In fruit juice industries, a fruit juice preparations having low viscosity and high clarity is looked for trait. To achieve these traits, pectinases plays a key role by liquefaction (of fruit pulps by depectinization). For apple juice, pectinase treatment augments the transmittance by 143% and reduces the viscosity substantially (Praveen and Suneetha 2016).

Depectinization reactions are generally carried out in the pH range of 2.0 to 6.0 at 40-50°C for a variable time-period ranging from 5 minutes to 6 hours. For example, in citrus juice processing PDEs help in removal of cloudiness and in stabilization of juices. Moreover, cell wall degrading enzymes like cellulases and hemicellulases are also added to release the pectin from plant cell wall (Wikiera *et al.* 2015).

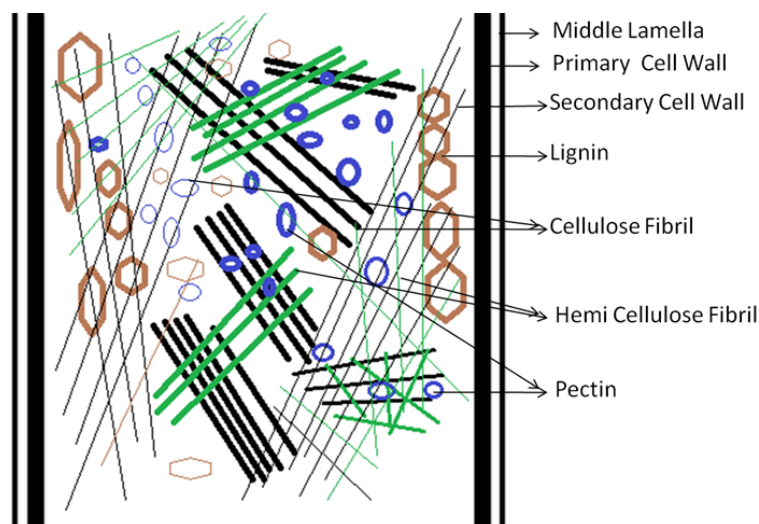


Fig. 1. Compositional Model of a typical plant cell wall. It depicts the location and importance of pectin in cell wall of the plant. Different components are labeled

Application of enzymes is also related to fruit types. Soft fruits like banana, carrot and papaya results in gel like mess because of high content of pectin. In this particular case, pectinases is essential for improving the pressing characteristics. Indeed, addition of PDEs increases the overall juice-yield by 3-4 folds. In pineapple juice preparations, addition of pectinase with hemicellulases enhances the juice yield by 25% (Tapre and Jain 2014; Garg *et al.* 2016).

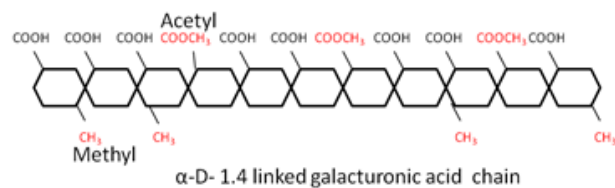
Pectinase treatment not only reduces the viscosity and enhances the clarity in juice preparations but also increase the release of polyphenols and phenolic components from fruit skin. These polyphenols has a proven role as an antioxidant that has many health benefits. Dragon

fruit juice obtained by enzymatic treatment has 15% more phenolics. So, PDEs also improves nutritional value of fruit juices (Tapre and Jain 2014).

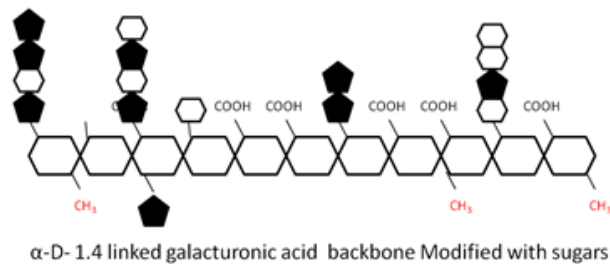
Wine making and processing

Fruit juices are the starting material for ethanolic fermentation. Pre-treatment of fruit juice enhance the ethanol yield, sensory characteristics of wine and stability of fermented products. In some case, pectinase treated wine has methanol because of the action of pectin/pectate methyl esterase (PME). Therefore, it is highly recommended that the pectinase enzyme mixture used in wine mixture must contain either no or a very restricted amount of PMEs (Hoondal *et al.* 2002).

Homogalacturonan (HG)



Rhamnogalacturonan II (RG-II)



Rhamnogalacturonan I (RG-I)

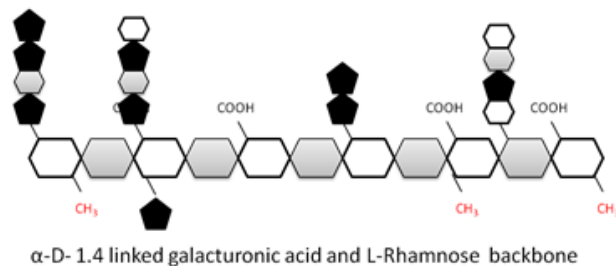


Fig. 2. A schematic diagram of the three major types of pectins– HG (Homogalacturonan), RG-I (Rhamnogalacturonan I), RG-II (Rhamnogalacturonan II)

Biomass hydrolysis and Biofuel

Pectin-rich biomass as sugar beet pulp, citrus waste, and apple pomace has shown promise as bioenergy feedstocks to produce bioethanol. Noticeably, these bioenergy feedstocks will require saccharification and fermentation methods that are optimized for the suite of sugars they contain and a tailored or engineered *E. coli* has been shown to use the pectin rich biomass for bioethanol

production. Enzymatic hydrolysis of the feedstock is prerequisite for the pectin rich biomass refinery where the role of pectin degrading enzymes is central (Turner *et al.* 2007).

Although, in cellulosic biomass the pectin contributes a small amount but in a recent study it has been reported that the addition of pectate lyases in cellulase enzyme mixture reduces the enzyme loading by approximately 25-30%. Hence,

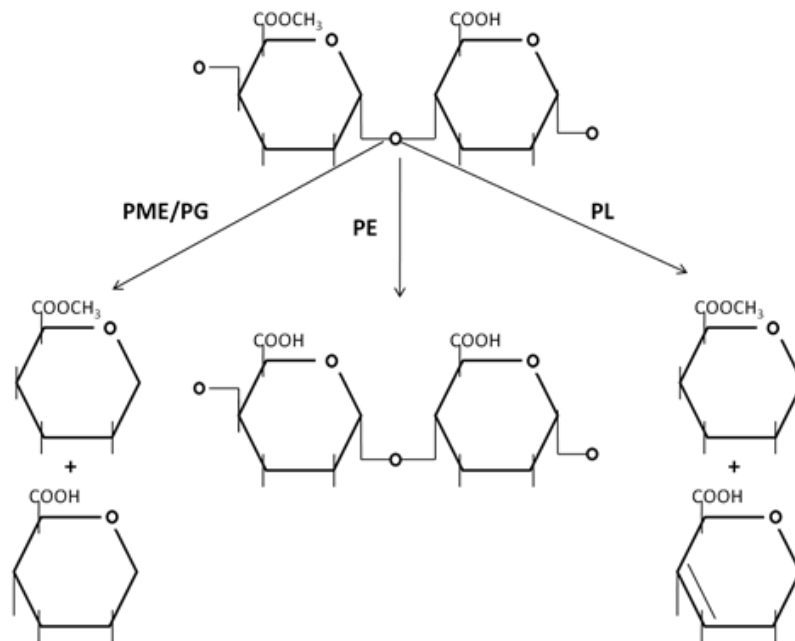


Fig. 3. A schematic diagram explaining three broad categories of pectin (Homogalacturonan, HG) degrading enzymes: (1) PMG/PG – polymethylgalacturonase or polygalacturonase; (2) PE – pectin esterase and (3) PNL/PL – Pectin lyases or pectate lyases

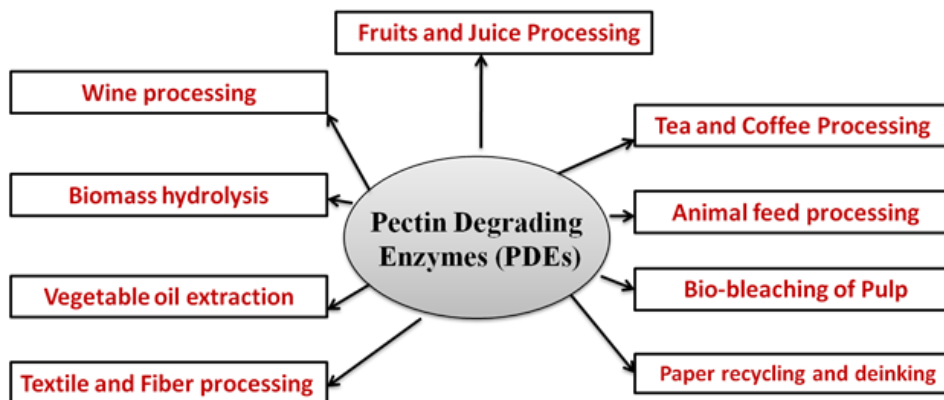


Fig. 4. A drawing highlighting the applications of pectin degrading enzymes (PDEs) in different industries

the PDEs is also very effective in development of a cost-effective cellulase mixture for cellulosic ethanol (Xiao and Anderson 2013).

Extraction of vegetable oil

Vegetable oils of olive, sunflower, coconut, palm or canola are nowadays extracted with the use of alkaline pectolytic enzymes together with an enzyme concoction having cellulases, hemicellulases. Enzymatic treatment increase oil yield as well as boost the polyphenolic and vitamin E content. In summary enzyme based extraction of oil from oil seed plants is a green process and role of PDEs step up the yield and nutritional value of extracted oil (Kusuma and Sri Rami Reddy 2014).

Processing of textile material

Bio-scouring is a method for removal of non-cellulosic impurities from the fiber and cotton materials. Chemical methods are currently being replaced by eco-friendly enzymatic process. Alkaline pectinase has been found as the most appropriate enzyme for cotton scouring by many researchers but enzyme combinations like lipase alongside pectinase is more effective for bio-scouring of cotton fibre. Since last decade, the feasibility of enzymatic scouring to cotton, jute, coir, flax, hemp, ramie and banana has been attempted with success (Garg *et al.* 2016).

Tea and coffee Processing

The reduction in pectin content of tea leaves by pectinase treatment speed up the tea fermentation and reduces the foam forming property of instant tea powder and develops a characteristic aroma. Similarly, the removal of the mucilage layer of coffee bean improves the yield, reduction in the processing time, aroma and flavour of robusta coffee (Garg *et al.* 2016).

Processing of Animal Feed

Animal feeds are very rich in fibre and inclusion of cellulolytic, xylanolytic and pectinolytic enzymes enhance the food absorption in the rumen gut by lowering down the viscosity of the feed. This was quite evident in increase of animal weight upon inclusion of PDEs in the animal feed.

Biobleaching of the Kraft Pulp

The presence of pectins causes yellowness of paper in the sheet formation for the paper and pulp industry. On chemical bleaching the kraft pulp brightens the paper sheet by reducing the galacturonic acid content. The same reduction

has been also achieved by enzyme and thus use of pectinase in the biobleaching is growing now these days. In fact, synergistic action of xylanase with pectinase in biobleaching of pulp has shown more promise in processing of kraft pulp (Kirk *et al.* 2002; Srivastava and Malviya 2011; Garg *et al.* 2016).

De-inking and Paper recycling

Deinking process is a key step for paper recycling and at present use of chemicals in the above process throws up a big environmental challenge for waste effluent treatment. Thus, the enzymatic de-inking by using a mixture of pectinases, hemicellulases, cellulases and lignolytic enzymes is more preferred. Enzymatic deinking modifies the bonds near the ink particle and remove ink from fibre surface. Subsequently, the released ink takes off by washing or floatation. Again, a combination of xylanase and pectinase has been successfully used for development of an eco-friendly process of the deinking of waste papers (Kirk *et al.* 2002; Srivastava and Malviya 2011; Kusuma and Sri Rami Reddy 2014; Li *et al.* 2014; Garg *et al.* 2016).

CONCLUSION

Conclusively, PDEs have diverse applications centred on food and beverage industries as well as a potential application in area of bioenergy for biorefinery based on pectin rich feedstocks. A new research report by Global Market Insights has predicted the growth of industrial enzymes market is expected to cross USD 9.5 billion by 2024; and the major growth has been speculated for food & beverages and animal feed industry where pectin degrading enzymes alone have share of approximately 20-25% (Kumar *et al.* 2014; Liu *et al.* 2016). Thus, studies related to pectin degrading enzymes are a very active research area especially for thermostable pectin degrading enzymes. These thermostable enzymes will have major impact on fruit and vegetable processing, paper and pulp industry and in bioenergy. At present Commercially, Novozyme Inc is sole supplier of thermostable pectinase – Novozym 33095 which has a considerable stability up to 60°C and the optimum activity is in a range of 50-60°C (Sharma *et al.* 2013; Priya and Sashi 2014). So, novel and new thermostable pectinases

are currently being sought to meet the growing demand.

However, in literature there are only a few reports of thermostable pectinase such as a PGase from *Streptomyces* sp. QG-11-3 is 60 °C (Beg *et al.* 2000), a pectinase from a hyperthermophilic bacterium *Thermotoga maritima* having temperature optima of 80°C (Klusken *et al.* 2003; Pijning *et al.* 2009), a thermostable pectinase from *Bacillus halodurans* M29 having the similar temperature optima and a pectate lyase from *Caldicellulosiruptor bescii* with temperature optima in the range of 85-90°C. Hence, to meet the demand and better understanding of structure and functions of PDEs, the study of thermostable pectin degrading will be a hot topic for the research and many biotechnological applications (Turner *et al.* 2007; Pedrolli *et al.* 2009; Kusuma and Sri Rami Reddy 2014; Su *et al.* 2015).

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