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REVIEWS

Microbial pectinase: sources, characterization and applications

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Abstract Today pectinases are upcoming industrially important bacterial enzymes. It can be produced by a variety of microorganisms. These enzymes act on pectin, which is the major component of middle lamella in plant cell wall. Pectinolytic enzymes are classified according to their mode of attack on the galacturonan part of the pectin molecules such as protopectinases, esterase's and depolymerases. As we know that microbial enzymes work depends up on the type of enzymes application, temperature, concentration, and pH and so on, therefore, pectinase enzyme also differentiated according to their physical and chemical factors too. The biochemical structures of pectinases include members of all the major classes and the structure–function relationship, studies of a few available complexes of pectinases with substrate/analogs could be considered as prototypes for related family member and the molecular characterization of pectinolytic enzymes is also well documented. Furthermore, it provides a bird's eye view of the possible application of these enzymes in commercial sector.

Keywords Pectin · Pectinase · Pectinolytic bacteria · Microbial pectin

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1 Introduction

Enzymes are the bio-active compounds that regulate many chemical changes in living tissues. Pectinases are a heterogeneous group of related enzymes that hydrolyze the pectin substances, present mostly in plants. Pectinolytic enzymes are widely distributed in higher plants and microorganisms (Whitaker 1991). They are of prime importance for plants as they help in cell wall extension (Jayani et al. 2005) and softening of some plant tissues during maturation and storage (Sakai 1992; Aguilar and Huirton 1990). According to the cleavage site, pectinases are divided into three groups: (1) hydrolases consisting of polygalacturonase, PG (EC 3.2.1.15); (2) lyase/trans-eliminases comprising pectinlyase, PNL (EC 4.2.2.10), and pectate lyase, PL (EC 4.2.2.2); (3) pectin esterase, PE (EC 3.1.1.11) (Yadav et al. 2009; Visser et al. 2004). Therefore, pectinase enzymes are commonly used in processes involving the degradation of plant materials, such as speeding up the extraction of fruit juice from fruit, including apples and sapota. Pectinases have also been used in wine production since the 1960s (Semenova et al. 2006). It can solve the undesirable problem of spoilage and decay of the processed foods, by the treatment or use of efficient pectinases which can degrade homogalacturonan and rhamnogalacturonic acid of pectin to convert it into sugar and other useful compounds. These pectinolytic enzymes catalyze the degradation of pectin substances which is of great industrial importance.

2 Substrate for pectinase enzyme

Pectin is the versatile and structurally homo polysaccharide contained in the middle lamella and primary cell walls of terrestrial plants, occupying one-third of the dry weight of plant tissue (Gupta et al. 2008). Pectin containing long galacturonic acid chains with residues of carboxyl groups and with varying degree of methyl esters (Voragen et al. 2009). One of the most studied and widely used commercial pectinases is polygalacturonase. It is useful because pectin is the jelly-like matrix which helps cement plant cells together and in which other cell wall components, such as cellulose fibrils, is embedded. It was isolated and described by Braconnot et al. (2006). It is produced commercially as a white to light brown powder, mainly extracted from citrus fruits, and is used in food as a gelling agent particularly in jams, jellies, sweets and as a stabilizer in fruit juices and milk drinks and as a source of dietary fiber (Fig. 1).

3 Origin and occurrence of pectinase

Pectinase activity is implicated in cell wall metabolism including cell growth, fruit ripening, abscission, senescence and pathogenesis (Gaffe et al. 1997; Dorokhov et al. 1999). Commercially PE can be used for protecting and improving the texture and firmness of several processed fruits and vegetables as well as in the extraction and clarification of fruit juices (Fayyaz et al. 1993). PE is found in plants, plant pathogenic bacteria and fungi (Fayyaz et al. 2003). It has been reported in *Rhodotorula* sp. (Libkind et al. 2004), *Phytophthora infestans* (Forster 1988), *Erwinia chrysanthemi B341* (Pitkanen et al. 1992), *Saccharomyces cerevisiae* (Gainvors et al. 1994), *Lachnospira pectinoschiza* (Cornick et al. 1994), *Pseudomonas solanacearum* (Schell et al. 1994), *Aspergillus niger* (Maldonado et al. 1994; Maldonaldo and Saad 1998), *Lactobacillus lactis* subsp. *Cremoris* (Karam and Belarbi 1995), *Penicillium frequentans* (Kawano et al. 1999), *E. chrysanthemi 3604* (Laurent et al. 2000), *Penicillium occitanis* (Hadj et al. 2002), *A. japonicas* (Semenova et al. 2003), others. There are many reports of occurrence of PE in plants, viz., *Carica papaya* (Fayyaz et al. 1993; Innocenzo and Lajalo 2001), *Lycopersicum esculentum* (Warrilow et al. 1994), *Prunus malus* (Macdonald and Evans 1996), *Vitis vinifera* (Correding et al. 2000), *Citrus* sp. (Arias and Burns 2002), *Pouteria sapota* (Arenas-Ocampo et al. 2003) and *Malpighia glabra* L. (Assis et al. 2004).

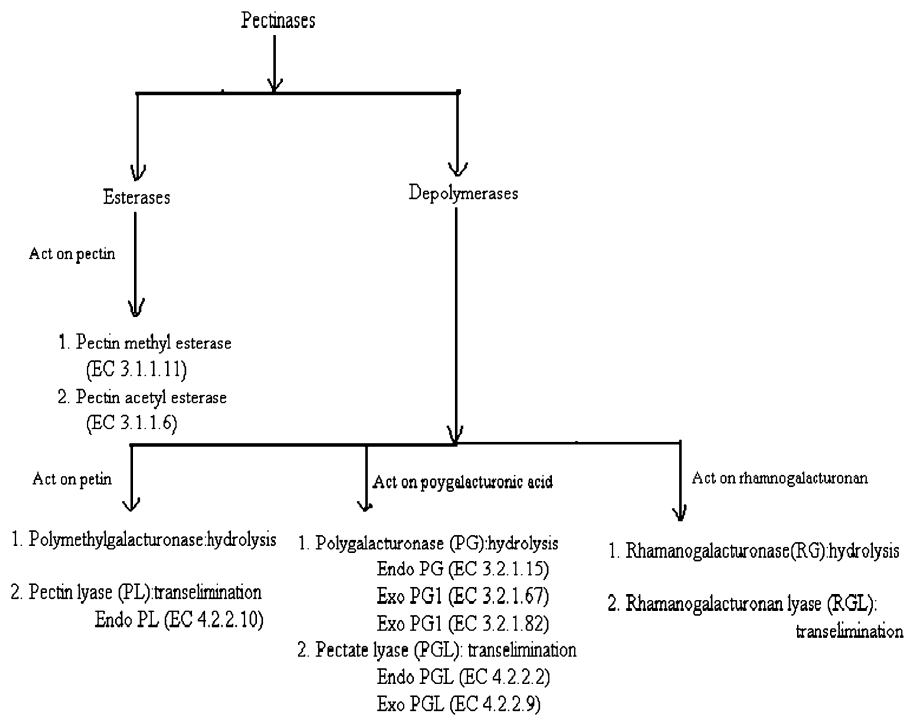

Fig. 1 Classification of different pectinases based on their reaction with different pectin substances

4 Mode of action of pectinase

It has been estimated that microbial pectinases account for 25 % of the global food enzymes sales. Microbial pectinases can be produced by many organisms like bacteria, actinomycetes, yeast and fungi. Protopectinases, polygalacturonases, lyases and pectin esterase's are among the extensively studied pectinolytic enzymes. As shown schematically in Fig. 2 (Alkorta et al. 1998) that protopectinases catalyze the soluble protopectin, polygalacturonases hydrolyze the polygalacturonic acid chain by addition of water and are the most abundant among all the pectinolytic enzymes. Lyases catalyze the trans-eliminative cleavage of the galacturonic acid polymer. Pectin esterase's liberate pectin and methanol by desertification in methyl ester linkages of the pectin backbone (Sharma et al. 2011).

Pectinases are one of the most important groups of enzymes used in fruit and vegetable industry for increasing juice yield and juice clarification (Sharma et al. 2011). These enzymes act on pectin, pectinolytic enzymes are classified according to their mode of attack on the galacturonan part of the pectin molecules. They can be distinguished from pectin methyl esterase's, that de-esterify pectin's to low methoxyl pectin or pectic acid and from pectin depolymerases, which split the glycosidic linkages between galacturonosyl (methyl ester) residues. Polygalacturonases split glycosidic linkages next to free carboxyl groups by hydrolysis while pectate lyase split glycosidic linkages next to free carboxyl groups by β-elimination. Both endo types of polygalacturonases (PGs) and pectin lyase (PLS) are known to randomly split the pectin chain. Exo-polygalacturonase releases monomers or dimers from the non-reducing end of the chain, whereas exo-pectin lyases release unsaturated dimers from the reducing end (Martin et al. 2004). Highly methylated pectins are degraded by endo-pectin lyases and by a combination of pectin esterase's with polygalacturonase (Sarkenen 1991).

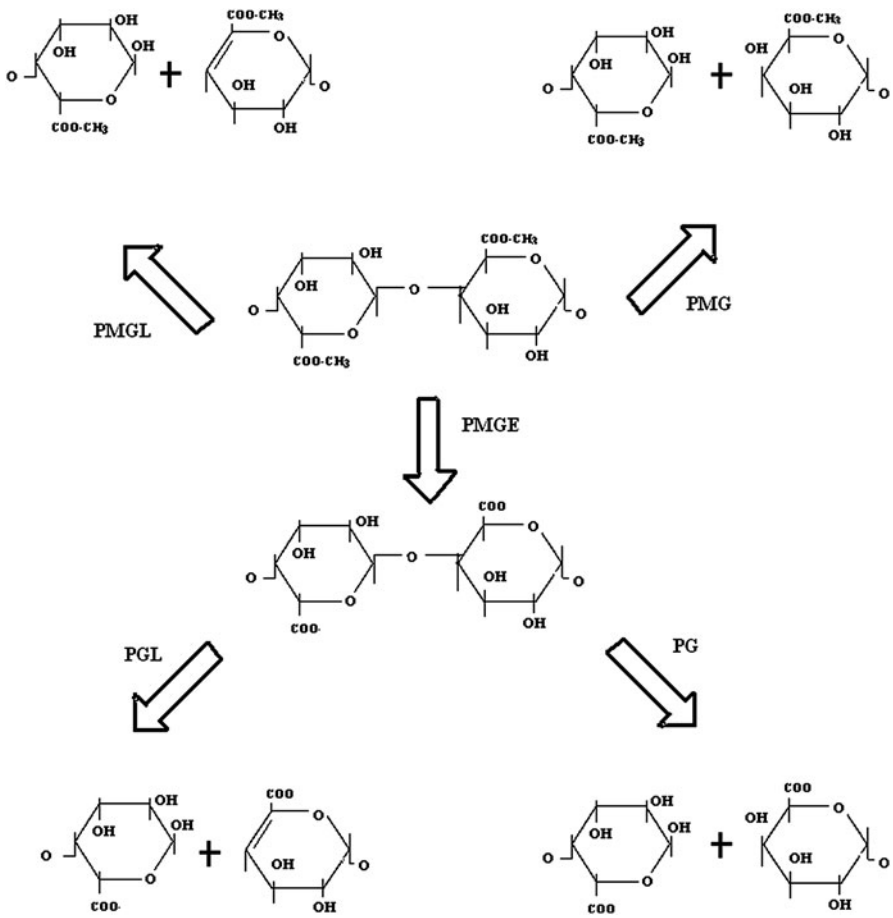

Fig. 2 Enzymatic mode of action of PMGL, PMG, PGL and PG on the pectin molecule. *PMGL* polymethylgalacturonate lyase (pectin lyase), *PMG* polymethyl galacturonase (pectin hydrolase), *PMGE* polymethyl galacturonate esterase (pectin esterase), *PG* polygalacturonase (pectin hydrolase)

5 Physicochemical characterization

Microbial enzymes work depends up on the type of enzymes application, temperature, incubation time, agitation, concentration, pH and use of different enzyme preparations (Dominguez et al. 1994; Chadha et al. 2003). Therefore, pectinase enzyme also differentiated according to there physical and chemical factors such as:

5.1 Polymethyl galacturonase (PMG)

Polymethyl galacturonase works on optimum pH between 4 and 5 and highly esterified pectin (95 %) was the best substrate (Schnitzhofer et al. 2007) for it.

5.2 Polygalacturonases (PG)

Endo PGs often occur in different forms having molecular weights in the range of 30–80 kDa and p*I* ranging between 3.8 and 7.6. Most endo PGs have their optimum pH in the acidic range of 2.5–6.0 and an optimum temperature of 30–50 °C (Singh and Appu-Rao 1989; Takao et al. 2001). Exo PGs are widely distributed in *A. niger*, *Erwinia* sp. and in some plants such as carrots, peaches, citrus and apples (Alonso et al. 2003; Pathak and Sanwal 1998). The molecular weight of exo PGs vary between 30 and 50 kDa and their p*I* ranges between 4.0 and 6.0.

5.3 Pectin lyase (PL)

The molecular mass of PL lie in the range of 30–40 kDa (Soriano et al. 2005; Hayashi et al. 1997) except in the case of PL from *Aureobasidium pullulans* and *Pichia pinus* (~90 kDa). In general PL has been found to be active in acidic pH range of 4.0–7.0 although some reports show PL activity even in alkaline conditions (Sakiyama et al. 2001; Moharib et al. 2000). Isoelectric point has been found to be in the range of 3.5 for PL. The K_m values for PL are in the range between 0.1 and 5 mg/ml respectively depending on the substrate used (Sakiyama et al. 2001; Moharib et al. 2000). The thermal deactivation of PL from *A. niger* was modeled by first- kinetics and found that the deactivation rate constant is minimum at pH 3.9 and 29 °C (Naidu and Panda 2003) and the optimum pH and temperature are 4.8 and 35 °C, respectively (Naidu and Panda 1999).

5.4 Polygalacturonate lyase (PGL)

The molecular weight of PGL varies between 30 and 50 kDa except in the case of PGL from *Bacteroides* and *Pseudoalteromonas* (~75 kDa) (McCarthy et al. 1985; Truong et al. 2001). The optimum pH lies between 8.0 and 10.0 although PGL from *Erwinia* and *Bacillus licheniformis* were active even at pH 6.0 and 11.0 respectively.

5.5 Pectin esterase or pectin methyl esterase (PE)

The molecular weight of most microbial and plant PEs varies between 30 and 50 kDa (Hadj et al. 2002; Christensen et al. 2002). The optimum pH for activity varies between 4.0 and 7.0 except for PE from *Erwinia* whose optimum pH is in alkaline region. Most PE has optimum temperature in the range 40–60 °C and a p*I* varying between 4.0 and 8.0. Industrially PE can be used to maintain the texture and firmness of processed fruit products and in clarification of fruit juices.

6 Biochemical structure of pectinase

Three-dimensional structures of pectinases (Fig. 3a, Mayans et al. 1997; Fig. 3b, Jensen et al. 2010) enable understanding of the molecular basis of enzyme mechanism, the role of individual amino acids in the active sites and also provide a rationale for structural differences between the enzymes that lead to very specific recognition of unique oligosaccharide sequences from a heterogeneous mixture in the plant cell wall. Crystal structures of pectinases include members of all the major classes and the structure–function relationship studies of a few available complexes of pectinases with substrate/analogs could be considered as prototypes for related family members.

The first crystal structure of a pectinase was that of *Erwinia chrysanthemi* pectate lyase C (PelC) (Yoder et al. 1993). The same structural fold has subsequently been observed in other members of the pectinase family. These include additional pectate lyases, *E. chrysanthemi* PelA (Thomas et al. 2002), PelE (Lietzke et al. 1994) and Pel9A (Jenkins et al. 2001); *Bacillus subtilis* Pel (Pickersgill et al. 1994)and high alkaline pectate lyase (Akita et al. 2000); two pectin lyases from *Aspergillus niger*, PLA (Mayans et al.

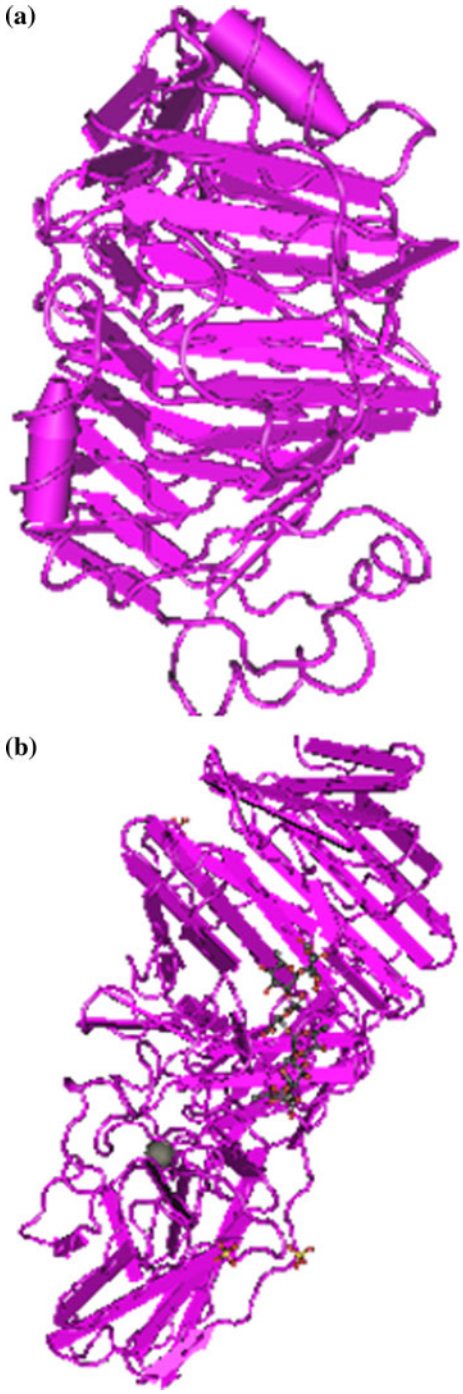

Fig. 3 Three dimensional structure **a** pectin lyase A, **b** rhamnogalacturonan lyase from Aspergillus *aculeatus* K 150a substrate complex

1997) and PLB (Vitali et al. 1998); polygalacturonases, *Erwinia carotovora* polygalacturonase (Pickersgill et al. 1998), *A. niger* endopolygalacturonase I and II (Van Pouderoyen et al. 2003; Van Santen et al. 1999) *Aspergillus aculeatus* polygalacturonase (Cho et al. 2001), *Stereum purpureum* endopolygalacturonase I (Shimizu et al. 2002) and *Fusarium moniliforme* endopolygalacturonase (Federici et al. 2001); pectin methylesterases from *Daucus carota* (Johansson et al. 2002), *Lycopersicon esculentum* (Jenkins et al. 2001)and PemA from *E. chrysanthemi* (Di Matteo et al. 2005)and *Aspergillus aculeatus* rhamnogalaturonase A. (Petersen et al. 1997).

Each structure consists of a single domain of parallel β-strands folded into a large right-handed cylinder. The domain fold, termed the parallel β-helix, is compatible with all accepted structural rules, albeit in a unique manner. The central cylinder consists of seven to nine complete helical turns and is prism shaped due to the unique arrangement of three parallel β-strands in each turn of the helix. The strands of consecutive turn's line up to form three parallel β-sheets called PB1, PB2 and PB3. PB1 and PB2 form an antiparallel β sandwich, while PB3 lies approximately perpendicular to PB2. Although the mechanism of pectin cleavage differs for the esterase's, hydrolases and lyases, the substrate binding sites as deduced from structures, sequence similarity and site directed mutagenesis studies (Kita et al. 1996), are all found in a similar location within a cleft formed on the exterior of the parallel β-helix between one side of PB1 and the protruding loops. The structural differences in the loops are believed to be related to subtle differences in the enzymatic and maceration properties. There is presently no structure of a representative from the PMG family.

6.1 Polygalacturonases

The endo PGs are inverting glycosidases that invert the anomeric configuration of the products during the reaction. In this mechanism, the hydrolysis proceeds by a general acid catalyst donating a proton to the glycosidic oxygen and a catalytic base guiding the nucleophilic attack of a water molecule on the anomeric carbon of the galacturonate moiety bound at the -1 subsite. The crystal structures of native *S. purpureum* endo PG I and that of the ternary product complexes with two molecules of galacturonate provided experimental evidence of the substrate binding mechanism, active site architecture as well as the reaction mechanism (Shimizu et al. 2002). In site-directed

mutagenesis studies of *A. niger* endo PG II, the replacement of charged residues His195, Arg226, Lys228, and Tyr262 led to tenfold or greater increases in the K_m value(Armand et al. 2000; Pages et al. 2000). Thus confirming the importance of the carboxyl group recognition in sub site +1 for productive substrate binding. In contrast, the replacement of Asp173 caused only a twofold increase in the K_m value, but greatly decreased the K_{cat} value (Armand et al. 2000) Asp173 is expected to serve as a general acid catalyst that donates a proton to the glycosidic oxygen. Thus, the tight binding of the substrate to sub site +1 is due to the electrostatic interactions between the carboxyl group and the basic residues and the precise recognition of the galactose epimer. This probably accounts for the fact that endo PGs are able to cleave only free polygalacturonate and not the methyl esterified substrate (Shimizu et al. 2002).

6.2 Polygalacturonate lyase (PGL)

All proteins in the PGL family are believed to share a similar enzymatic mechanism. The enzyme randomly cleaves pectates by a β-elimination mechanism, generating primarily a trimer end-product with a 4,5-unsaturated bond in the galacturonosyl residue at the non-reducing end (Petersen et al. 1997). The β-elimination reaction in pectinolytic cleavage involves three steps: (a) neutralization of the carboxyl group adjacent to the scissile glycosidic bond, (b) abstraction of the C5 proton, and (c) transfer of the proton to the glycosidic oxygen. Among the structures of members of the PGL superfamily, the Michaelis complex of a catalytically inactive R218K PGL mutant, Pel C from *E. chrysanthemi* and a plant cell wall fragment (a penta Gal*p* A substrate) reveals important details regarding the enzymatic mechanism (Scavetta et al. 1999). Structural studies of Pel C at various pH and Ca^{2+} concentrations have shown that Ca^{2+} binding is essential to the in vitro activity of Pel C and that the Ca^{2+} ion has multiple functions (Pickersgill et al. 1994; Herron et al. 2003). The Ca^{2+} ions link not only the oligosaccharide to the protein but also adjacent uronic acid moieties within a single pectate strand. The observed Ca^{2+} positions are very different from the inter strand Ca^{2+} ions postulated to link PGA helices together (Walkinshaw and Arnott 1981a, b; Jayani et al. 2005).

Identification of common coordination of the −1 and +1 subsite saccharide carboxylate groups by a protein-liganded Ca^{2+} ion, the positioning of an arginine catalytic base in close proximity to the α-carbon hydrogen, numerous other conserved enzyme–substrate interactions and mutagenesis data suggest a common polysaccharide *anti-β*-elimination mechanism for both families (Brown et al. 2001). The absence of sequence homology between distinct families of pectate lyases suggests that such catalytically similar enzymes have evolved independently and may reflect their different functions in nature.

6.3 Pectin lyase (PL)

Enzymatic cleavage in PL occurs via the same β-elimination reaction as seen for PGLs. However, in contrast to PGL, PL are specific for highly methylated forms of the substrate and do not require Ca^{2+} for activity. Crystal structures available for the apo-forms of pectin lyase A (Mayans et al. 1997) and pectin lyase B (Vitali et al. 1998) from *A. niger* show that both adopt the parallel β-helix fold and are structurally almost identical. Comparison of structures of PGL and PL show that although they share many structural features, there appears to be remarkable divergence in the substrate binding clefts and catalytic machinery reflecting differences in their substrate specificities. The divergence in substrate specificity comes from two factors. Firstly, the loops making the active side cleft in PL is much longer and of a more complex conformation that encompasses two β-strands forming an antiparallel β-sheet. Secondly, the putative active site of PL exhibits a cleft that is predominantly aromatic comprising of four tryptophans and three tyrosines that contribute to the architecture of the active site. Although these enzymes share a common fold and related mechanisms, their strategies for substrate recognition and binding are completely different (Mayans et al. 1997).

6.4 Pectin methyl esterase

Pectin esterases catalyzes pectin de-esterification by hydrolysis of the ester bond of methylated α-(1-4)-linked D-galacturonosyl units, producing a negatively charged polymer and methanol. Among the PE structures are those of *E. chrysanthemi* PemA and a PE from carrot. They both have similar structures and

belong to the family of parallel β-helix proteins with major differences in loops making up the substrate binding cleft. The putative active site of PE was deduced from mapping of sequence conservation among PEs onto the structure. The substrate binding cleft is located in a location similar to that of the active site and substrate binding cleft of PGL and PL. The central part of the cleft is lined by several conserved aromatic amino acids, especially on the exposed side of PB1. The active site of PE is located in the long shallow cleft lined by two absolutely conserved aspartic acid residues in the center, Asp136 and Asp157 in carrot PE. The following mechanism of action has been suggested for carrot PE (Johansson et al. 2002). Asp157 acts as the nucleophile for the primary attack on the carboxy methyl carbonyl carbon while Asp136 may act as an acid in the first cleavage step, where methanol is leaving. Asp136 could then act as a base, in the next step extracting hydrogen from an incoming water molecule to cleave the covalent bond between the substrate and Asp157 to restore the active site.

7 Molecular characterizations of pectinase

The molecular characterization of pectinolytic enzymes is well documented. Numerous studies on fungal pectinolytic enzymes have been carried out and several fungal PNL genes have been isolated and characterized from *Aspergillus niger* (Gysler et al. 1990; Someren et al. 1991 and Someren et al. 1992), *A. oryzae* (Kitamoto et al. 2001), and *Glomerella cingulata* (Templeton et al. 2001). A number of PEs have been purified and biochemically characterized (Whitaker 1991). Crystal structures of pectin lyase A (PNLA) from two strains of *A. niger*, N400 and 4M-147 (Mayans et al. 1997), reveal that PNLA folds into a parallel b-sheet and shares many of the structural features of PL despite not more than 17 % sequence identity after pair-wise structure-based alignment. These shared structural features include amino acid stacks and an asparagine ladder. The substrate-binding clefts of these two PNLs are dominated by aromatic residues and are enveloped by negative electrostatic potential. The major difference between these two PNLA structures is in the conformation of the loop formed by residues 182–187. These observed differences are due to the different pH values of crystallization. The three dimensional structure of pectin lyase B (PNLB) from *A. niger* has also been determined by crystallographic techniques at resolution of 1.7 Å (Vitali et al. 1998). The multiple sequence alignment of different PNL protein sequences from different organisms can provide us an opportunity to design degenerate primers for PCR amplification of PNL gene family, which can further be used for cloning and over expression.

All the sequences of PNL, PL, PG, and PE of different source organisms available in GenBank from NCBI.A total of 494, 717, 937, and 172 protein sequences of PNL, PL, PG, and PE, respectively, represent major groups of pectinases. Only the full length protein sequences were considered for in silico analysis. The program Clustalw (Lassmann and Sonnhammer 2006) and Seaview was used for multiple sequence alignment. Mega 3.1(Kumar et al. 2004) was used for dendrogram construction by neighbor-joining (NJ) (Salemi and Vandamme 2003), Minimum Evolution, and UPGMA methods (Shi et al. 2007). For domain search, the Pfam site was used. Domain analysis was done using MEME. The conserved protein motifs deduced by MEME were subjected to biological functional analysis using protein BLAST, and domains were studied with Interproscan providing the best possible match based on highest similarity score.

To determine which site is the pectinolytic active site and what function of the second site might be, many site-specific mutants of PelC have been prepared and characterized. In all mutants, the maceration activity on plant tissue parallels the observed pectinolytic activity, supporting the widely-held hypothesis that pectate cleavage is responsible for the observed "soft rot" damage, because Ca^{2+} is required for in vitro pectinolytic activity (Aalbersberg et al. 2003).

As shown in Fig. 1 pectinases is further divided into Esterase's and Depolymerases. In esterase's the enzymes are able to do hydrolase's that splits esters into an acid and an alcohol with liberate water in a chemical reaction is called hydrolysis. Depolymerases are subdivided into trans-elimination and hydrolysis.

7.1 Polymethylgalacturonase (PMG)

Polymethyl galacturonase activity can be determined by measuring the reducing sugars formed due to the hydrolysis of glycosidic bond or by measuring the

reduction in viscosity of the substrate. Few reports are available on the biochemical characteristics of the enzyme due to the following reasons: (a) the enzyme has not been purified to homogeneity and characterized and (b) the activity of this enzyme has not been demonstrated in the absence of the other pectin enzymes and in the presence of 100 % methylated pectin. *Aspergillus* was found to be a major producer of PMG followed by species belonging to *Penicillium*, *Botrytis* and *Sclerotium*. PMG from *A. niger*. A highly acidic pectinase (optimum pH 2.3) from *A. niger* has also reported (Naidu and Panda 1998).

7.2 Polygalacturonases (PG)

Polygalacturonase hydrolyzes the glycosidic linkages of polygalacturonates (pectates) by both exo and endo splitting mechanisms. PG activity can be determined by measuring the reducing sugars formed due to hydrolysis or by viscosity reduction method. However, the viscosity reduction method is less sensitive for exo PGs as the decrease in viscosity is relatively low. Cup plate method can also be used for estimating PG activity by viewing the clearing zones after staining with ruthenium red (Truong et al. 2001). Endo PGs are widely distributed among fungi, bacteria and yeast.

7.3 Pectin lyases (PL)

Endo-PL degrades pectic substances in a random fashion yielding 4:5 unsaturated oligomethylgalacturonates and exo-PL has not been identified so far. Trans-eliminative pectin de-polymerization was first demonstrated by using pectin lyase from *A. niger* (Van Alebeek et al. 2002). The measurement of viscosity reduction can also be used to measure the activity of PL but is predominantly used to determine whether the enzyme is endo or exo-splitting. Pectin lyases do not show absolute requirement of calcium for its activity except for *Fusarium* PL. However, it has been reported that PL activity can be stimulated in the presence of calcium (Soriano et al. 2005; Hayashi et al. 1997).

7.4 Polygalacturonate lyase (PGL)

Endo-PGL and Exo-PGL are reported to degrade pectate by trans-elimination mechanism yielding 4,5 unsaturated oligogalacturonates. PGLs are found only in micro-organisms and they have an absolute requirement of calcium ions for activity. PGLs have an optimum pH near alkaline region (6–10), which is much higher than other pectinases (Singh et al. 1999; Truong et al. 2001; Dixit et al. 2004).

7.5 Pectin esterase or pectin methyl esterase (PE)

Pectin esterase hydrolyzes the methoxy groups from 6-carboxyl group of galacturonan backbone of pectin. The product of degradation of pectin by PE is pectic acid, methanol and a proton from the ionization of newly formed carboxyl group. Pectin esterase's are primarily produced in plants such as banana, citrus fruits and tomato and also by bacteria and fungi (Hasunuma et al. 2003). PE is more specific towards highly esterified pectin substances and shows no activity towards pectates. PE activity increases with increase in degree of esterification of substrate.

8 Applications of pectinase

Application of pectinolytic enzyme is varied according to availability of physical conditions. Over the years, pectinases have been used in several conventional industrial processes, such as textile, plant fiber processing, tea, coffee, oil extraction, treatment of industrial wastewater, containing pectinacious material, etc. They have also been reported to use in purification of viruses and in making of paper. The first commercial application of pectinases was observed in 1930 by Kertesz. Pectinases in spite of their usage in other industries are endowed with promising applications in fruit processing industry as juice clarifies, color and yield enhancers and in fruit mash treatment (Junwei et al. 2000). Abundant waste from agriculture and fruit processing industrial waste becomes an appreciable section for biological utilization of fruit processing waste in juice industry. Pectinases play a crucial role in clarification (Kobayashi et al. 2001), extraction, in reduction of viscosity, to remove off the peels and to increase the yield (Sanchez and Demain 2002). In apple, pear and grape pectinases are used during pressing and straining stages. In Orange, Mango, Guava, Pineapple and Papaya pectinases are employed to remove the cloudiness.

Pectinases are of prompt application in maceration, liquefaction (Kashyap et al. 2001), extraction

(Bhat 2000; Pilnik and Voragen 1993), cloud stabilization (Grassin and Fauquembergue 1996; Rebeck 1990), clarification processes (Kareem and Adebowale 2007; Alkorta et al. 1998) and gelation (Rajagopalan and Krishnan 2008). Pectinolytic enzymes added to macerated fruits before the addition of wine yeast in the process of producing red wine resulted in improved visual characteristics (color and turbidity) as compared to the untreated wines. Enzymatically treated red wines presented chromatic characteristics, which are considered better than the control wines. These wines also showed greater stability as compared to the control (Jayani et al. 2005).

8.1 Textile processing and bio-scouring of cotton fibers

Pectinases have been used in conjunction with amylases, lipases, cellulases and hemicellulases to remove sizing agents from cotton in a safe and ecofriendly manner, replacing toxic caustic soda used for the purpose earlier. Bio-scouring is a novel process for removal of non-cellulosic impurities from the fiber with specific enzymes. Pectinases have been used for this purpose without any negative side effect on cellulose degradation (Jayani et al. 2005).

8.2 Degumming/retting of plant bast fibers

Alkaline pectinase are mainly used in degumming and retting of fiber crops. Pectinolytic enzymes are involved in the retting and degumming of jute, flax, hemp, ramie, kenaff (*Hibiscus sativa*), and coir from coconut husks (Chesson 1980; Bruhlman et al. 1994). Retting is a fermentation process, in which certain bacteria (e.g., *Clostridium*, *Bacillus*) and certain fungi (e.g., *Aspergillus*, *Penicillium*) decompose the pectin of the bark and release fiber (Sharma and Robinson 1983). Commercially, retting is done by one of the two basic forms. In dew retting (an aerobic process) plant straw is thinly spread on the ground and exposed to the action of the fungi and aerobic bacteria for 2 ± 10 weeks. Species of *Cladosporium, Penicillium, Aspergillus* and *Rhodotorula* have been isolated from dew-retted plants (Cao et al. 1992).

For degumming mostly neutrophillic microbes have been studied so far (Bruhlman et al. 1994) but due to difficulties in prevention of heterogeneous contamination and some other disadvantages, these strains have not been applied on an industrial scale. In a series of experiments carried out by (Cao et al. 1992), they have reported the isolation of a Bacillus sp. (strain NT-33) which could grow well at pH 10.0, which is of great advantage to the productivity and activity of degumming enzymes. Moreover, fermentation at high pH values also has the advantage of preventing contamination and thus an open fermentation process can be developed. This strain has been reported to remove more than 70 % ramie gun after 24 h fermentation under alkaline conditions. The quality of degummed fibers produced by this technique satisfied the textile industry standards. Pectinolytic enzymes from actinomycetes have also shown good correlation between the pectate lyase activity and the degumming effects, resulting in good separation of the bast fiber.

8.3 Waste water treatment

The wastewater from the citrus-processing industry contains pectinaceous materials that are barely decomposed by microbes during the activated-sludge treatment (Tanabe et al. 1986). Vegetable food processing industries release pectin, containing wastewaters as by-product. Pretreatment of these wastewaters with pectinolytic enzymes facilitates removal of pectinaceous material and renders it suitable for decomposition by activated sludge treatment (Jayani et al. 2005).

A soft-rot pathogen, *Erwinia carotovora* (FERM P-7576), which secrets endo-pectate lyase, has been reported to be useful in the pretreatment of pectinaceous wastewater (Tanabe and Kobayashi 1987). However, because of the danger from its phytopathogenicity, indirect pretreatment by the pectinolytic enzyme produced from the bacterium has also been compared and reported to solubilize almost all the pectin substances contained in the wastewater.

8.4 Coffee and tea fermentation

Fermentation of coffee using pectinolytic microorganisms is done to remove the mucilage coat from the coffee beans and to enhance the tea fermentation and foam forming property of tea (Soresen et al. 2000). Fungal pectinases are also used in the manufacture of tea. Enzyme treatment accelerates tea fermentation, although the enzyme dose must be adjusted carefully to avoid damage to the tea leaf. Large-scale treatment

of coffee with commercial pectinases is costly and uneconomical, inoculated waste mucilage is used as a source of microbial pectin enzymes. The fermentation liquid is washed, filtered and then sprayed on to the beans (Soresen et al. 2000).

8.5 Paper and pulp industry

Pulp and paper mills are beginning to use enzymes to solve problems in their manufacturing processes. Alkaline pectinase produced by *Bacillus* sp. and *Erwinia carotovora* (Kotoujansky 1987; Palomaki and Saarilahti 1997), due to its strong macerating activity, has been used for retting of Mitsumata bast (Tanabe and Kobayashi 1987). These retted basts have been used for the preparation of Japanese paper (Horikoshi 1990). During papermaking; pectinase can depolymerise pectins and subsequently lower the cationic demand of pectin solutions and the filtrate from peroxide bleaching. In modern papermaking, retention aids are added to pulp to keep fines and filler particles in paper sheets and to speed the drainage of water. Cationic polymers of various structures are commonly used as retention aids (Horn and Linhart 1996). Alkaline peroxide bleaching of pulps solubilizes polysaccharides, which are troublesome interfering substances (Holbom et al. 1991). Pectinase can depolymerize polymers of galacturonic acids, and subsequently lower the cationic demand of pectin solutions and the filtrate from peroxide bleaching (Reid and Ricard 2000).

8.6 Animal feed

Pectinases are used in the enzyme cocktail, used for the production of animal feeds. This reduces the feed viscosity, which increases absorption of nutrients, liberates nutrients, either by hydrolysis of non-biodegradable fibers or by liberating nutrients blocked by these fibers, and reduces the amount of faeces (Jayani et al. 2005).

8.7 Purification of plant viruses

In cases where the virus particle is restricted to phloem, alkaline pectinases and cellulases can be used to liberate the virus from the tissues to give very pure preparations of the virus (Jayani et al. 2005).

8.8 Oil extraction

Oils from rape seed (Canola), coconut germ, sunflower seed, palm, kernel and olives are traditionally produced by extraction with organic solvents. The most commonly used solvent is hexane, which is a potential carcinogen. Recently, the plant cell-wall-degrading enzyme preparation has begun to be used in olive oil preparation. The enzymes are added during grinding of the olives, thereby the oil is released easily in the subsequent separation techniques (West 1996). Improvement of chromaticity and stability of red wines.

Pectinases have a wide role in control of disease causing pathogenic microbes, though pectin does not seem to be the most recalcitrant cell wall component, the pectin degrading enzymes have proved to be decisive in the infection process, so long as pectin degradation results in weakening of cell walls, leading to penetration of fungi (Ten Have et al. 2002). Generally, fungal pathogens secrete various extracellular enzymes and may be involved in virulence (Wanyoike et al. 2002; Wubben et al. 2000). Positive effects were shown for pectinolytic enzymes from *Aspergillus flavus* (Shieh et al. 1997), *B. cinerea* (Ten Have et al. 1998) and *Claviceps purpurea* (Oeser et al. 2002). The disruption of genes encoding these enzymes resulted in the reduction of virulence in the respective fungi. The precise role of most other extracellular enzymes is still controversial (Voigt et al. 2005).

In most studied cases correlations were observed between the presence of pectic enzymes, disease symptom and relative virulence. An interesting example is observed in *B. cinerea*, where the endo-polygalacturonase (PG) activity is regulated by at least 5 endo-PG genes, and the deletion of only one endo-PG gene reduced virulence on tomato (Ten Have et al. 1998). The expression on transgenic plants of a PG inhibitory protein resulted in a considerable reduction of virulence of *B. cinerea*. Similar reduction of virulence was measured with PG mutants from *B. cinerea* on wild-type tomato (Powell et al. 2000). Yakoby et al. (2001) showed that heterologous expression of a pectate lyase from *Colletotrichum gloeosprioides* in *Colletotrichum magna* led to increase virulence of transformants on watermelon.

In *C. purpurea* replacement of two closely linked PG genes resulted in a sharp reduction of pathogenicity

on rye, but the disruption of other CWDE genes (celluloses, xylanases) did not have effect on virulence significantly (Oeser et al. 2002). On the other hand, a double mutant of *C. carbonum* lacking the two major extracellular PGs (having less than 1 % of total wild-type PG activity) displayed normal virulence on maize (Scott-Craig et al. 1990).

9 Commercialized preparation of microbial pectinase

Several microbial pectinases have been commercialized for various industrial applications. *A. niger* (Perrone et al. 2006; Tjamos et al. 2004; Abe et al. 1988), and *B. subtilis* (Koboyashi et al. 1999, Singh et al. 1999, Takao et al. 2000) are the major sources of pectinase enzymes at commercial level such as Novo Nordisk Pectinex™, Pectinex SP-L (Maiorano et al. 1995), Pectinex 1XL, Pectinex SP and so on these are some example of commercialized pectinase which are used and supplied by Carolina Biological Supply Company, CCM International Ltd. and many more industries (Sutton and Peterson 2001).

The conditions for submerged production of PG and its purification were partially optimized. Owing to the expensive medium ingredients, solid state fermentation (SSF) was tried using banana peel, wheat bran, sugarcane bagasse, orange bagasse as a carbon source separately and in different combinations in order to find out cheap and suitable natural source for production of this industrially important pectinase enzyme.

The wide range commercial application of Pectinases in the market, enhancement of production technology in Bio-chemical aspects, detailed understanding of the fermentation processes and various recovery methods made the microbial production of enzymes affordable (Janssens et al. 1992; Gummadi and Panda 2003). Decreasing the capital investment by using low cost agricultural and fruit processing industrial waste as raw materials not only booms up the commercialization of the product but also aids in recycling (Patill and Dayanand 2006). A multi-step process involving screening of soil samples from agro-industrial wastes for native novel (Horikoshi 1995; Patill and Chaudhari 2010). Bacteria(organism), organized fermentation processes (up streaming and down streaming), implementation of techniques like strain improvement or any modern techniques to boost up the yield of isolated bacteria is to be employed in a coordinated way for successful large scale production of Pectinases.

Many microorganisms can be degrade the polymers to simple sugars by secreting extra cellular enzyme such as amylases, cellulases and pectinases (Bruhlman et al. 1994). Thus the enzyme technology is more focused on research on pectinolytic bacteria and on genetic modifications for production of high yielding strains of pectinolytic bacteria (Suneetha and Khan 2011).

Pectinolytic bacteria are the cheap and easily available source for the environmental management. Therefore, the work on pectinase will be accepted world wide. Some branch of science doing work on it such as bioinformatics can also play a major role in recent advent of research. It paves a simple way for structural analysis (Suneetha and Khan 2011).

10 Conclusion

Bacterial pectinase enzymes are classified under EC 3.3.1.15 and EC 4.2.2.2 category. Pectinase of natural origin could use as safer and cheaper alternative of existing synthetic industrial and medical processes. Presence of characteristics bacteria with pectinolytic activity has been reported from different types of sources but isolation from remote areas like agro-wastes could allow identification of novel strains. However, further characterization of this enzyme for utilization in agro-waste management and some other applications yet to be done.

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