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MINI REVIEW

Recent advancements in the production and application of microbial pectinases: an overview

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Abstract The wide utility and catalytic efficiency of microbial pectinase in various industries has greatly increased its global demand. Among the natural sources of pectinases, microbial pectinases are used frequently for its ease of production and unique physicochemical properties. Yet similar to other industrial enzymes, pectinases also face the constraint of thermo-tolerance and low yield in its economised production. The current review addresses the various strategies adopted to meet the high yield and thermo-tolerance of pectinases as well as the various attempts made in the field of pectinases to its improved production and better catalytic efficiency. The utilisation of natural as well as recombinant microbial sources, metagenomic approaches, metabolic engineering, site directed mutagenesis and media engineering techniques adopted in the field of pectinases have been discussed. The significance of pectinases in various industries is depicted by enlisting its applications. To the best our knowledge the current review is unique being the first attempt to compile the recent advancements in the field of pectinases.

Keywords Pectinase · Thermo-tolerance · Microbial · Polygalacturonase

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1 Introduction

Pectinases traced back as the first enzyme used in homes to its commercialized production since 1930, accounts for a major proportion of the industrial enzymes. They have attained more value with the multitude applications such as production of functional foods (Khan et al. 2013; Prathyusha and Suneetha 2011), retting and degumming of fibres in textile industry (Cao et al. 1992), production of good quality paper (Ahlawat et al. 2008), fermentation of coffee and tea, oil extractions and treatment of pectic waste water, bioethanol production etc. (Kashyap et al. 2001). The term pectinase refers to heterogeneous enzymes including homogalacturonan-degrading polygalacturonases (PG) or pectin depolymerase; polymethylgalacturonases (PMG); lyases or transeliminases and pectin esterases (PE), which is also known as pectin methyl esterases (PME).These enzymes are capable of either lysing the glycosidic bonds, debranching or modifying pectin, the most

abundant component of fruits (Cuesta 2016). Pectin functions as a cross linking polysaccharide in the primary cell wall and middle lamella of fruits and vegetables cross linking cellulose and hemicellulose fibres and the use of pectinases improve access of cellulases to their substrates (Giacobbe et al. 2014). Pectin hydrolases are produced mainly by fungi, being more active on acid or neutral medium at temperatures between 40 and 60 °C, whereas bacterial pectinases are more active in acidic conditions (Pedrolli et al. 2009). As per reports available approximately 75% of the industrial enzymes are hydrolases, with carbohydralases being the second largest group (Priya and Sashi 2014).

Pectinases are classified based on their mode of action into polygalacturonase (EC 3.2.1.15), Pectin esterase (EC 3.1.1.11), Pectin lyase (EC 4.2.2.10) and Pectate lyase (EC 4.2.2.2). These enzymes act on O-α-(1,4) poly galacturonopyranose structures with activities and specificities that depend partly upon the degree of methylation (Saadoun et al. 2013). Based on their cleavage specificity pectinases can be grouped into ones cleave pectin smooth regions or pectin hairy regions (Pedrolli et al. 2009). Protopectinases solubilizes protopectin and forms soluble pectin, pectin methyl esterases and pectin acetyl esterases eliminates methoxyl and acetyl residues from pectin which give rise to polygalacturonic acid, polygalacturonase breaks the glycosidic α-(1–4) bonds between galacturonic residues by hydrolysis and trans-elimination reactions. Pectinases are divided as acidic or alkaline based on pH; whereas they are termed as endo or exo when enzyme action is random or at terminal end respectively.

A critical study on the various reviews on pectinases indicate different aspects of this group of enzymes such as its structural–functional characteristics (Gummadi et al. 2007), purification (Gummadi and Panda 2003), applications (Khan et al. 2013; Pedrolli et al. 2009; Sharma et al. 2013) etc. The current review lays down the various attempts made in the field of pectinases to its increased production, improved thermo-tolerance and better catalytic efficiency of this enzyme. The increasing global demand of pectinases could be addressed by the adoption of either natural or recombinant enzymes by strain improvement, genetic engineering, metagenomic studies, site-directed mutagenesis, directed evolution and media engineering.

2 Significance of pectinase thermo-tolerance and enzyme yield

Most of the commercially available pectinases are combinations of pectate lyases, polygalacturonases and pectin methyl esterases mainly derived from *Aspergillus* sp (Kashyap et al. 2001). Of these acidic pectinases are widely used in food industry, while alkaline pectinases find applications in a variety of industrial processes. A close study of these enzymes reveal that these enzymes are exposed to extreme processing conditions with temperatures ranging from 30 to 70 °C in the food industry, for instance in sugar extraction and temperatures higher in biotech industries (Singh et al. 1999). A comparative analysis on the thermal inactivation of commercial pectinases revealed that some of the brands lost half of its activity on exposure to 50 °C for even 2 min of exposure (Ortega et al. 2004). Thus the choice of pectinases with better thermo-tolerance becomes essential for its effective utilization. Apart from tolerating high temperatures, thermo-stable enzymes would be advantageous in having a little activity at lower temperatures, longer shelf life, resistant to organic solvents, high and low pH solubility, less viscous and high reaction rates (Kusuma and Sri 2014). Moreover, by the use of thermo- tolerant enzymes the chance of enzyme inactivation during attempts to kill contaminating pathogens becomes quite rare.

The high yield and thermo-tolerance of pectinases can be addressed by use of natural or recombinant high yielding isolates in combination with efforts using metagenomic, metabolic engineering, site-directed mutagenesis and various production optimization strategies. Deep insights on the molecular characterisation, functional analysis, genetic studies and mutation strategies have been some of the key factors contributing to pectinase enzyme yield, its thermo-tolerance and alkaline stability.

2.1 High yielding natural strains

Pectinase is naturally present in plants and in fruits for natural ripening of fruits, yet microbial sources are commonly used for large scale production for application level studies and for industrial use due to ease of maintenance and production. Pectinolytic organisms can be isolated from spoiled fruits walls, soil, decaying

agro-waste, animals etc. The predominantly pectinase producing microorganisms include *Pseudomonas, Xanthomonas*, *Erwinia, s*oil isolates such as *Actinomycetes* and *Streptomycetes* and various fungi. *Aspergillus niger, Aspergillus versicolor, Aspergillus flavus, Fusarium oxysporum, Rhizopus stolonifer, Mucor racemous, Mucor hiemalis, Penicillium jenseni, Penicillium citrinum* and *Trichoderma viride* are the main fungal source of pectinases (Priya and Sashi 2014). Fungal organisms *A. niger* is a good source of pectinase. *A. niger* IM 6 gives maximum enzyme activity at 40 °C with 60% moisture on seventh-day solid state fermentation (Akhter et al. 2011). Almost 82% of pectinase activity was retained by *A. niger* strain MCAS2 pectinase even at 100 °C and the enzyme was found to be stable even at alkaline conditions (Khatri et al. 2015). Table 1 enlists some of the natural thermo-stable pectinases.

Thermostable pectinases have widely reported in various studies and these natural strains are selected when higher temperatures of processing are required. A comparative analysis shows that the optimal temperature for a Polygalacturonase from *Streptomyces* sp. QG-11-3 is 60 °C and the hyper-thermophilic bacterium *Thermotoga maritima* is at 80 °C (Kluskens et al. 2005). Most predominantly *Bacillus* sp. are good sources of thermo-stable enzymes (Rebello et al. 2017). The thermal stability of polymethyl galacturonase from thermo-stable *Bacillus* sp. BR1390 indicated 100% activity at 60 °C after 60 min and a residual activity of 50% at 90 °C was observed after 30 min of incubation (Banafshe and Hamid 2014).Various studies indicate that the thermo-stability is attributed to the presence of cysteine residue present in the amino acid sequences of pectinases enabling the formation of disulphide bonds as well as conferring a strong hydrophobicity to the pectinase (Singh et al. 2012a, b; You et al. 2010).

2.2 Recombinant pectinase sources

Recombinant pectinases also find various applications in foods and quite often the natural non-GMO food products also may be finally treated with recombinant pectinases to improve food quality. The most common source of recombinant pectinase used in the food industry includes mainly fungal derived pectinases such as *Aspergillus, Penicillium* and *Trichoderma* varieties as per GMO compass reports (http://www.gmo-compass.org/eng/database/ enzymes/92.pectinase.html). *Bacillus* derived recombinant pectinases are used predominantly in industrial purposes.

The use of highly efficient pectinases with peculiar properties under the promoter control of various expression hosts has been done in *Escherichia coli, Pichia pastoris, Saccharomyces cerevisiae*, *P. griseoroseum* have been reported as shown in Table 2. A comparative analysis of the yield, thermo-stability and pH tolerance of these enzymes helps greatly to validate their use in different fields. Recombinant expression of thermo-alkaline Pel (BacPelA) gene from *Bacillus clausii* in *E. coli* resulted in recombinant mature BacPelA with an enzyme activity of 8378.2 U ml^{-1} (A_{235}) by high-cell-density cultivation in fed-batch fermentation with productivity of 239.4 U ml^{-1} h^{-1} and this represents the highest Pel yield reported to date (Zhou et al. 2017). In another study recombinant *Aspergillus* expressed in *P. pastoris* achieved a maximal activity of 2408.70 U ml^{-1} in the culture supernatant of by high cell density batch fermentation, equivalent to a 4.8 times greater yield than that from shake-flask culture (Abdulrachman et al. 2017). *P. pastoris* based expression of an acid stable endo-polygalacturonase gene from *Penicillium oxalicum* produced a yield of 1828.7 U ml^{-1} (Cheng et al. 2017).The long lag time in basal salts medium (BSM) and an occurrence of proteolysis associated with recombinant pectinase production in *P. pastoris* KM71 has been found to be overcome by using synthetic FM22 medium for inoculum and proteolysis control by growth at lower pH (Charoenrat et al. 2013).

Recombinant *P. griseoroseum* T20 produced by transformation of *P. griseoroseum* with the plasmid pAN52*pgg*2, containing the gene encoding PG of *P. griseoroseum*, under control of the *gpd* promoter gene from *Aspergillus nidulans* yielded 266- and 27-fold greater levels of pectin lyase (PL) and polygalacturonase (PG) respectively than the wild-type strain (Teixeira et al. 2011). The use of a constitutive promoter such as the promoter of gpd gene instead of the indigenous promoter of *Penicillium* greatly enhanced the pectinase production. Attempts in the generation of catabolite repression resistant mutant strains of *Penicillium griseoroseum* mutants by UV-induced spontaneous mutations resulted in a 7.8-fold increase of pectinase production than the wild strain (Lima et al. 2017).

Table 1 Natural high pectinase yielding microbes

Microorganism	Type of pectinase	PH tolerance	Thermal stability	Enzyme activity	Reference
Bacillus subtilis ZGL14		pH stable at 8.6–10.0	Residual activity of 51, 40 and 22% when incubated at 80 °C for 60, 80 and 100 min	52,372.52 U/mg	Yu et al. (2017)
Sclerotium rolfsii	Polygalacturonases PG1 and PG2	Optimally active at pH 5	PG1 had $t_{1/2}$ of 20 min at 70 °C and $t_{1/2}$ of 80 min at 50 °C	300 U/ml	Schnitzhofer et al. (2007)
Pectobacterium carotovorum subsp. carotovorum BR1	Pectate lyase	Optimum at alkaline pH	Alkaline pH and Ca^{2+} ions favor thermostability of purified PL at 60 and 70 °C	–	Maisuria and Nerurkar (2012)
Penicillium notatum	Polygalacturonase on wheat bran	Optimum pH range of 4.5 to 6.0	Optimum activity at 50 °C	1129.62 U/gds	Amin et al. (2013, 2017b)
Penicillium occitanis	Pectate lyase with apple pectin	Optimum pH 9.0	Optimum 60 °C	434 U/ml	Damak et al. (2011)
Aspergillus fumigatus *Fres.* MTCC 4163	Pectinase and polygalacturonase (PG) using wheat bran substrate	pH 4.0 optimum denatured at higher and lower pH	Lost 25% activity on incubation at 80 °C for 10 min	Maximum of 1116 Ug^{-1} by pectinase and 1270 Ug^{-1} by polygalacturonase	Phutela et al. (2005)
Aspergillus fumigatus R6	Polygalacturonase solid state fermentation using rice bran	Broad range pH 3.0–8.0	–	565 U/g	Wong et al. (2017)
Aspergillus kawachii	Two inducible exopolygalacturonase	Optimum between 3.0–4.0	Exo PG1 at pH 4.0 after a 2 h incubation at 37, 50, a and 60 °C had residual activity of 95, 79 and 54%	45 U/mg-ExoPG1 32 U/mg-ExoPG2	Byrne et al. (2017)
Aspergillus niger	Pectin lyase liquid media containing 1% pure pectin	Retained 60–90% activity over the range 6.2–9.2	Stable up to 70 °C and about 82% of pectinase activity was still observed at 100 °C	Maximum activity of 24 U/ml at 2.5% pectin concentration	Khatri et al. (2015)
Aspergillus giganteus	Polygalacturonase (PG) on liquid Vogel medium with citrus pectin	Optimum pH 6.0, very stable over a neutral ,and alkaline pH range	Optimum 55–60 °C, half-life of 115, 18, and 6 min at 40, 50 and 55 °C, respectively	419.4 U/mg on purification	Pedrolli and Carmona (2010)
Aureobasidium pullulans	Polygalacturonase under submerged culture using tomato pomace agro-waste	Broad pH range (5.0–10.0)	Stability over a range of temperature (5–90 ◦ C) with an optimum at 60 °C	25.75 U/mL	Bennamoun et al. (2016)
Acrophialophora nainiana	Growth on 0.5% (w/v) pectin from citrus fruits	Optimum pH 8.0	Temp optimum of 60 °C, half-life of 7 days at 50 °C	286.63 nkatal/mg	Celestino et al. (2006)

Table 1 continued

Microorganism	Type of pectinase	PH tolerance	Thermal stability	Enzyme activity	Reference
Thermomucor indicae-seudaticae	SSF conditions on media containing a mixture of wheat bran and orange ba gasse (1:1) at 70% of initial moisture	Retained above 80% of maximum activity in the pH range from 3.0 to 10.0	the PG from SmF showed an increase of activity when incubated for 1 h at temperatures over 40 °C, reaching 120% of the initial activity after 1 h at 60 °C, while the enzyme from SSF lost 80% of its activity at 60 °C	SSF 120 U/ml of exo-PG, while in submerged fermentation (SmF) it produced 13.6 U/ml	Martin et al. (2010)
Thermoascus aurantiacus	Pectin lyase (Pl) and polygalacturonase (Pg)	Pg and Pl had optimum activity at pH 5.0 and 10.5–11.0, respectively	Pg was stable in the acidic to neutral pH range and at 60 °C for 1 h, whereas Pl was stable at acidic pH and at 60 °C for 5 h	43 U/g for Pg using wheat bran and 40 U/g for PI using orange bagasse and sugarcane bagasse	Martins et al. (2002)

Table 2 Most important recombinant pectinases and their properties reported in the last 10 years

Parent microbe	Expression host	PH stability	Thermal stability	Enzyme activity	References
Bacillus clausii	*Escherichia coli*	Maximum activity at pH 10.5	Maximum activity at 70 °C	8378.2 U ml^{-1}	Zhou et al. (2017)
Paenibacillus sp. 0602	*Escherichia coli*	7.6–10.4	$t_{1/2}$ of 9 h and 42 h at 50 °C and 45 °C respectively	2060 U mg^{-1} on polygalacturonic acid (PGA)	Li et al. (2014)
Bacillus halodurans	*Escherichia coli* JM109 (DE3)	Stable from pH 9.5–10.5	$t_{1/2}$ of 90 min at 80 °C	K_m and V_{max} values of 4.1 g L^{-1} and 351 U mg^{-1} protein	Mei et al. (2013)
Bacillus licheniformis	*Escherichia coli*	Optimum 7.0 Active at a broad range	Optimum at 70 °C $t_{1/2}$ of 5 h at 60 °C	14 U/mlS	Singh et al. (2012a)
Paenibacillus campinasensis BL11	*Escherichia coli*	Optimum pH 10	Optimum at 50 °C, $t_{1/2}$ of 103 min at 70 °C and 288 min at 40 °C	1623 IU mg^{-1}	Ko et al. (2011)
Aspergillus aculeatus	*Pichia pastoris*	pH5.0 optimum	Optimal condition at 50 °C	2408.70 ± 26.50 U/mL on citrus pectin	Abdulrachman et al. (2017)
Penicillium sp. CGMCC 1669	*Pichia pastoris*	Stable at acidic pH with pH optimum at 3.5	90% of the activity at 35–55 °C and remained active even at 0 °C	–	Yuan et al. (2011)
Bispora sp. MEY-1	*Pichia pastoris*	Good stability at pH 2.0–7.0	Optimally active at 50 °C	$K_{m,app}$ and $V_{max,app}$ values for polygalacturonic acid were 1.25 mg/ml and 2526 μmol/min/mg, respectively	Yang et al. (2011)

2.3 Metagenomic approach

Metatranscriptomics studies of the rumen of a dairy cow revealed the presence of pectinase producing microbes such as *Bacteroides*; *Prevotella* sp., *Bacteroides* sp. and the *Ruminococcus* (Comtet-Marre et al. 2017); while studies in sheep previously indicated the pectinase genes of *Butyrivibrio, Prevotella, Bacteroides* and *Fibrobacter* (Yuan et al. 2012). Studies on a soil metagenome yielded an ORF of a pectate lyase similar to *Bacillus licheniformis* and it was expressed in *E. coli* (Singh et al. 2012a). As noted this enzyme worked at a broad range pH and temperature not requiring Ca^{2+} for its activity.

Recombinant expression of *pelB* gene a soil metagenome product in *E. coli* turned out to be good bioscouring agent in the textile pretreatment process (Wang et al. 2014). The further optimization of this recombinant in a 7L bioreactor resulted in the production of pectinase with activity of 1816.2 Uml^{-1}. New insights on the presence and role of multiple pectin degrading enzymes such as pectin lyase, polygalacturonase, galactosidase, arabinofuranosidase and rhamnosidase was reported from a thermophilic compost metagenome (Wang et al. 2016). The above paper was also unique making a first report on the role of actinomycetes in pectin degradation.

2.4 Metabolic engineering strategies

Protoplast fusion between complementary auxotrophic and morphological mutant strains of *P. griseoroseum* and *P. expansum* was induced by polyethylene glycol and calcium ions (Ca^{2+}) to obtain recombinant RGE27 with a threefold increase in polygalacturonase and 1.2-fold pectin lyase production than the parental strain (Varavallo et al. 2007). A semi rational approach based screening and comparative analysis on poly galacturonidase (PGL) production using first, 6 signal peptides (amyX, bpr, vpr, yvgO, wapA and nprE) in *Bacillus subtilis,* yielded a bpr directed efficient PGL secretory expression with a PGL titre to 313.7 U ml^{-1} (Zhang et al. 2013). Further optimization and use of strong promoter P43 and Shine–Dalgarno sequence increased PGL titre to 446.3 U ml^{-1}; whereas fed-batch studies in a fermenter yielded a titre of 632.6 Uml^{-1} with a productivity of 17.6 U ml^{-1} h^{-1}, which was the highest secretory production of PGL by the *B. subtilis* system.

2.5 Site-directed mutagenesis

Site-directed mutagenesis (SDM) research on pectate lyase was initially done to elucidate its mechanism of action and active site analysis. SDM studies on pelC and pelE pectate lyases of *Erwinia chrysanthemi* revealed that the active site included also the Ca^{2+} binding site (Jurnak et al. 1996). Mutagenesis studies revealed that the amino acids around the Ca^{2+} binding site are involved in the catalysis reaction of pectate lyase. Another study on the pectate lyase of *A. niger* revealed that the substrate binds to the enzyme as a Ca^{2+}-substrate complex, thus explaining the absolute requirement of pectate lyases for Ca^{2+}-ions (Benen et al. 2003).

Improvements in the thermo-stability of pectate lyase of *Xanthomonas campestris* origin (PL_{Xc}) was achieved by a single beneficial mutation (R236F) based on melting temperature guided sequence alignment; resulted in a 6 °C increase in T_m and a 23-fold increase in the half-life at 45 °C without compromising the enzymes catalytic efficiency (Xiao et al. 2008). Combination of R236F with another beneficial mutation (A31G) caused a hydrophobic desolvation of the enzyme with a two-fold increase in specific activity of the enzyme maintaining the improved T_m value.

Site-directed mutagenesis of polygalactauronidase of *S. cerevisiae* expressed in *P. pastoris* showed that aspartic acid residues at positions 179, 200, and 201 and histidine 222 were critical for enzyme activity (Blanco et al. 2002). Mutation of the two potential glycosylation sites at residues 318 and 330 revealed that double mutations at these two sites by converting asparagine to aspartate caused a 50% reduction in enzyme activity when compared to the wild-type *PGU1* transformant.

Directed evolution studies conducted on pectate lyase generated 12 mutants (A118H, A182 V, T190L, A197G, S208 K, T219 M, T223E, S255R, S263 K, N275Y, Y309 W, and S312 V) with thermo-tolerance greater than the parent strain (Solbak et al. 2005). However the best performing isolate had eight point mutations which contributed to a melting temperature 16 °C greater than the wild strain. Directed evolution of pectin methylesterase of *E. chrysanthemi* involving a four amino acid substitution led to the formation a mutant enzyme with T_m value of 11 °C from the wild type, maintaining its wild type kinetic properties.

2.6 Efforts to optimised industrial production

Pectinases are produced commercially by both submerged (SmF) and solid state fermentation (SSF), with the latter giving better enzyme yields. Various factors such as substrate selection, process conditions, water content, incubation time, inoculums size, pH, temperature, presence of inhibitors/activators and addition of carbon and nitrogen sources are crucially influencing the pectinase biosynthesis. A comparison on pectinase yield by 6 different fungus on different substrates viz, wheat bran, rice bran, orange peels, peanut shells, canola oilseed cake and sugarcane bagasse found wheat bran a promising substrate (Amin et al. 2017a). The role of ammonium sulphate in the induction of pectinase production was also evident with fungi such as *Aspergillus fumigatus* and *A. alliaceus* BIM-83 (Phutela et al. 2005; Sapunova et al. 1997). The former isolate *A. fumigatus* attained a maximum activity of 1116 Ug^{-1} for pectinase and 1270 Ug^{-1} for polygalacturonase at pH 4.0 and 5.0, respectively on growth on wheat bran (Phutela et al. 2005). Intermittent agitation exhibited a positive effect in the SSF of pectinase production in pilot scale bed reactor (Finkler et al. 2017).

Production of thermostable alkaline pectinases by *Bacillus pumilus* dcsr1 was increased 1.7-fold at optimized conditions with a 14.2-fold high enzyme production obtained in solid state fermentation than in submerged fermentation (Sharma and Satyanarayana 2012). An enzyme yield of 348 ± 11.8 Ug^{-1} was obtained on agro residue used as substrate moistened with mineral salt solution and optimum water activity was 0.92, optimum pH 9.0 and optimum temperature obtained was 40 °C.

An evaluation on the various bioreactors used in the production of pectinases indicate that better results were obtained by solid state fermentation as shown in Table 3. Pectinase production by *A. niger* LB-02-SF in a bench-scale rotating drum bioreactor indicated that enzyme production was favoured in conditions limiting the fungal growth, without any temperature control but with an intermediate air flow. Thus the best conditions for biomass growth were not the best for pectinase production (Poletto et al. 2017).

A novel strategy extraction protocol aiming to reduce the cost and increase the enzyme yield was adopted in the downstream process of pectinase extraction (Wolf-Marquez et al. 2017). The above extraction strategy utilised the salting-out potential of two biocompatible cholinium-based ionic liquids ($N_{1112}OHCl$ and $N_{1112}OHH_2PO_4$) in aqueous solutions of Tergitol, enabling 90% extraction of pectinase. Further on the exposure of pectinase to pulsed electric field was found to increase its thermal stability and activity without altering its structure (Zhang et al. 2017). Mild ultrasound treatment of pectinase increased its immobilization yield as well as its catalytic activity, but reduced its thermo-stability, reaction stability and reusability due to structural changes (Ma et al. 2017).

2.7 Structural and genetic factors contributing to enzyme properties

The presence of various conserved aminoacid residues in pectinases, the protein conformation and interacting bonding forces play a great role conferring to the properties such as thermotolerance, alkaline stability and catalytic efficiency to these enzymes. Polygalacturonases generally possess a conserved right-handed parallel β-helical structure with ten complete turns (Bonivento et al. 2008), with the active site open on both sides in endo-PGs or occluded on one side in the exo-PG (Abbott and Boraston, 2007). A unique tetrameric β-helical structure was also reported in exopolygalacturonase from *Thermotoga maritime,* the most thermotolerant pectinase reported so far (Pijning et al. 2009). The tetrameric nature of this protein accounts to its thermotolerance, substrate specificity (exo-activity and acceptance of non-methylated, saturated polygalacturonate only), as well as product specificity (release of mono-galacturonate).

Three conserved aspartate residues of pectinase interact with the substrate, with one residue (Asp173) acting as an acid, while the other two (Asp153 and Asp174) act as a base in the hydrolytic cleavage of the substrates (Pijning et al. 2009). Recent studies indicate that cation-π interactions of endo-polygalacturonases critically affect the thermotolerance and catalytic efficiency of these enzymes. Three single mutants of polygalacturonases viz, H58Y, T71Y and T304Y promoted the cation-π interactions of the enzyme, thereby increasing its thermostability (T_m increased by 0.6–3.9 °C) and catalytic efficiency to a 32-fold (Tu et al. 2016).

The genetic analysis of various pectinase coding genes in different microbes indicate that this enzyme could be coded by post-translational modification a polypeptide encoded by a single gene as in *Fusarium*

Table 3 An outline of various bioreactors used in pectinase production

Type of reactor	Principle	Substrate	Yield	References
Column-tray bioreactor	Solid-state fermentation	Lemon peel pomace	2181 U/L	Ruiz et al. (2012)
Pilot-scale packed-bed bioreactor	Solid-state fermentation	Wheat bran and sugarcane bagasse	1840 U kg^{-1} of dry solid per h	Pitol et al. (2016)
Pilot-scale packed-bed bioreactor	Solid-state fermentation	Wheat bran and sugarcane bagasse	22 U g^{-1}	Finkler et al. (2017)
Rotating drum bioreactor	Solid-state fermentation	Rice bran	4 U gdm^{-1}	Colla et al. (2016)
Tray-type reactor	Solid-state fermentation	Wheat bran	298 Ug^{-1} substrate	Demir and Tari (2017)
Stirred tank reactor	Submerged fermentation (yielded exopectinase and endopectinase)	Orange peel	Exo and endo: batch fermentation (670.7 and 28.2 U/L h, respectively) pulsed fed-batch (189.1 and 24.89 U/L h, respectively)	Wolf-Marquez et al. (2015)
Packed bed reactor (PBR) with recycled flow for continuous production	Solid-state fermentation	Spent grains	Volumetric productivity (P(V) = 0.98 U ml^{-1} h^{-1}	Almeida et al. (2003)

moniliforme (Caprari et al. 1993) or encoded by family of diverged genes as in *A. niger* (Bussink et al. 1992). Microarray analysis of *A. niger* grown on main sugar components of pectin revealed the expression of 46 pectolytic genes in the *i*solate (Martens-Uzunova and Schaap 2009) and most of these multigenes have been generated by gene duplication (Carroll et al. 2005).

Presence of substrates such as pectin, galacturonic acid and polygalacturonic acid induce the secretion of pectate lyase, polygalacturonase and pectin methyl esterase encoding genes, but glucose represses these genes. A clear involvement of carbon catabolite repressor protein (Cre A) is also found in the process of repression (Maldonado and Saad, 1998). The presence of various conserved sequence 5′-TYATTGGTGGAA-3′ and 5′-CCCTGA-3′ aiding in gene expression were identified in *A. niger* (Visser et al. 2004).

3 Applications of pectinases

Pectinases basically find applications both in acidic and alkaline conditions particularly in the food and textile industry respectively. Applications studies with pectinases are ongoing in global research fields to obtain maximum fastened activity with enzymes. The wide applications of this enzyme has attributed to its increasing global demand and some of these applications are reviewed here (Fig. 1).

3.1 Protoplast isolation

Protoplasts can be isolated by mechanical or enzymatic methods. Pectinases finds application for protoplast isolation in combination with other enzymes like cellulases and chitinases. Protoplasts have a wide range of applications in genetic transformations, membrane studies etc. An enzyme cocktail consisting of commercial cellulases, crude pectinases and crude chitinases was used to release maximum number of protoplast from *Pleurotus eous* and *P. flabellatus* mycelia (Parani and Eyini 2011). Mycelia extracted and incubated with enzyme cocktail for 3 h, KCl (0.6 M) as osmotic stabilizer, phosphate buffer of pH 6.0 and 3 days old culture gave maximum protoplast yield.

3.2 Fruit juice clarification

Fungal pectinolytic preparations are widely used for the clarification of fruit juices. Acid pectinases are

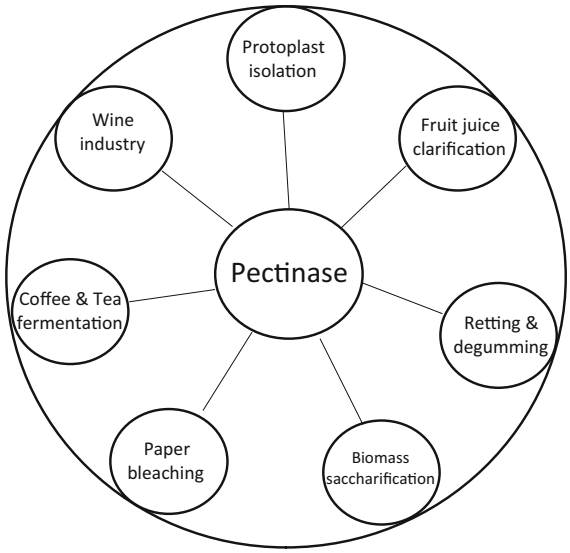

Fig. 1 Schematic presentation of various applications of pectinases

mainly used to remove pectin in fruit juice. Pectinases produced by *A. niger* (fungal pectinases) are commonly used to clarify fruit juices. Fruits are rich in pectin, crushing of fruit gives highly viscous fruit juice. Pectin is responsible for haze and precipitate formation in juice. The gelatinous nature of juice will result in clear juice extraction difficulties. Pectinase usages in extraction process improve juice quality. Pectinases increases filtration efficiency of fruit juice by decreasing turbidity of fruit juices (Saadoun et al. 2013). Pectinases degrades gel structure and decreases viscosity of juice. More than 90% improvement than mechanical extraction of clear juice and enzyme treatment also improves colour, flavour and nutritional characteristics of juice. Mainly used in apple juice preparation, clearness will be high when compared to pectinase untreated juice and on enzymatic treatment total pectin content of juice decreases (Pedrolli et al. 2009).

The benefits of using pectinases in juices include also the release of various phenolic compounds from fruits which serve as good antioxidants aiding in prevention of various ailments such as cancers and coronary heart diseases (Aliaa et al. 2010). Strawberry and raspberry juices need enzymatic de-pectinization to remove the colloidal nature (Versari et al. 1998). The pectin, phenolic substances and protein of juices result in formation of irreversible complexes that enzyme cannot break so it is essential to remove pectin. Pectinases depolymerize highly esterified pectin in apple juice. A combination of pectinase, cellulase and amylase gives juice yield up to 100% (Pasha et al. 2013). The enzyme treated banana can be used to produce banana wine, which gives cleared banana wine without affecting any other characteristics of banana wine (Tapre and Jain 2014).

3.3 Retting and degumming of fibre crops

Fibres containing gum should be degummed for its usage in textile industry. Pectin cements the fibres together and this pectin should be degraded. As chemical degumming causes pollution, an alternative use of pectinases and xylanases mixture serves as an eco-friendly and economical solution for pollution with non biodegradable pollutants (Sharma and Satyanarayana 2012). Alkaline pectinases are commonly used for retting and degumming process of fibres like ramie, flax, sunn hemp and jute. Pectinolytic enzymes secreted by soft rot bacteria cause maceration of woody fabrics that are long, strong and stiff, which softens fibres (Liao 1989).

Pectinase based enzyme retting is an eco-friendly process and has several advantages over water retting providing a high yield, quality and consistent quality to fibres. Moreover, it is a faster process and produces fewer odours. Green stem of ankra plant wet retting takes 10–12 days but 6% NaOH treatment with enzyme ankra twigs fibre extraction takes only 1 day. The use of medium twigs for enzyme treatment resulted in 81.5% pulp yield (Kuhad and Singh 2013).

In another study banana fibre separation was attained by using pectinase enzyme produced by *A. niger* (Azzaz et al. 2013). In higher plant tissues cells are united together through middle lamella are rich in pectin, on enzyme treatment middle lamella will be destroyed and separation of fibres can be observed. Scanning electron microscopy reveals that pectin degraded and fibres are separated. Apart from use in treatment of natural fibres, pectinase treatment was also found to increase the mechanical properties of reinforced thermoplastic composites (Saleem et al. 2008).

3.4 Animal Feeds

Reports on the supplementation of pectinases in animal feed suggest that it helps to increase the

absorption of nutrients by animals aided by degradation of fibres entrapping them (Hoondal et al. 2002).

3.5 Liquefaction and saccharification of biomass

Bioethanol obtained from enzyme treated biomass is a suitable alternative to fossil fuels and it helps to remove green house gas emissions. As the complex pectinaceous structures in feed stock degraded and hydrolysed by pectinases, the rate of ethanol production increases (Chen et al. 2012). Enzymatic hydrolysis of biomass is efficient treatment without generation of toxic waste and economically feasible process. Liquid hot water treatment enlarges the accessible and susceptible surface area of sugar and makes it accessible to hydrolytic enzymes. Substrate with enzyme cocktail of 2.5U gives high level of galacturonic acid released shows high percentage of saccharification.0.25–2.0 g lemon peel in 15 ml buffer with enzyme gives 94.59% saccharification and 1 g yield of sugar. Increase in lemon peel concentration decreases saccharification yield and reducing sugars and it may be due to feed back inhibition i.e. high end product (reducing sugar) concentration which results in enzyme inactivation (Mostafa et al. 2013).

3.6 Coffee and tea fermentation

Alkaline pectinases are generally produced by bacteria *Bacillus* species. Some fungi and yeasts also produces alkaline pectinases which are used for coffee and tea fermentation (Pedrolli et al. 2009). Soft ripe coffee fruits sorted and passed into mechanical pulper to remove coffee skin. Only coffee seed is released through pulper screen, thin viscous inner mesocarp which is a highly hydrated layer called mucilage can be removed by natural fermentation then washed dried to 35% moisture content.

Pectinase treatment increase tea fermentation rate and destroy foam forming property of instant tea powders by destroying pectin (Pasha et al. 2013). Pectinase from *A. niger, Byssochlamys fulva* and *Mucor circinelloids* used for fermentation of tea leaves from *Camellia sinensis* plant, increased production of phenolic compounds increases tea quality. Polygalacturonase are mainly used to increase tea quality (Thakur and Gupta 2012).

3.7 Wine industry

Pectinases are also widely used in wine making industry to increase the quality of wine (Rehman et al. 2015). The use of pectinases in combination of other enzymes such as hemicellulases, glucanases and glycosidases in the wine industry is considered to be the oldest application of this enzyme. The use of pectinase in wine industry in the grape must is done mainly to support the extraction process to maximize juice yield, facilitate filtration and intensify the flavour and colour (Sieiro et al. 2012). However, the use of pectinases is often low in commercial preparations to avoid the production of excess amount of methanol by the action of pectin methyl esterases.

3.8 Essential oil extraction

The utility of pectinase in the extraction of essential oils from various sources such as olives (Ortiz et al. 2017), flaxseed oil (Kulkarni et al. 2017), dates (Mehanni et al. 2017) etc. have been widely studied. Pectinase treatment yields oil of superior quality than organically extracted oil with lower fatty acids, peroxide value and colour intensity (Mehanni et al. 2017). Moreover the retaining of phospholipids in solid phase reduces the cost for refining process.

3.9 Paper bleaching, deinking and recycling

Pectinases in combination with xylanases are primarily used in the paper industry as a bio-bleaching agent. Unlike the conventional chemical bleaching agents, the use of enzymes is found to be eco-friendly, less harsh and good in improving the quality of the paper. Apart from reducing the kappa number and permanganate number of pulp, biological bleaching using pectinases in combination with xylanases brighten the paper and improve its physical properties (Kaur et al. 2010; Nathan et al. 2017). The replacement of chemical pectinases also contribute to reduce the chlorine disposal into the environment compared to those chemical alternative strategies. Recent studies indicate that ultra filtered concoction of pectinase and xylanase give better result than crude enzyme (Sharma et al. 2017). Biological deinking and bleaching would aid in lowering the BOD and COD of waste water before disposal (Singh et al. 2012a).

3.10 Others

The use of pectinase in the production of non-digestible oligosaccharides especially bioactive compounds has also been reviewed (Bezerra et al. 2017). The use of enzyme decoctions of pectinases and other enzymes are used to obtain good preparation of viruses from plant tissues (Butot et al. 2007). The role of pectinases in treatment of waste water is also evident in the remediation of water from pectin waste exuding industrial units (Singh et al. 2012a).This makes wastewater devoid of pectinaceous matter and ready for treatment by activated sludge treatment (Hoondal et al. 2002).

4 Conclusion and future perspectives

Application of pectinases in various fields shows the potential of green process. The various applications of pectinases as noted in the above section, greatly demands its economised production. Yet the properties of thermo-tolerance and high yield would surely be an advantage to these enzymes to its effective utilisation. Thus the use of various proteomic, genomic and production optimization methodologies either singly or combinatorial should be attempted to meet the increasing global demands of pectinases. Fine tuning will lead to the development of an economically viable strategy.

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