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REVIEW ARTICLE

INDUSTRIAL APPLICATIONS OF THERMOPHILIC PECTINASE: A REVIEW

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ABSTRACT

Pectin caused turbidity and viscosity interferes in fruit juice extraction while hydrophobicity causes problem in dyeing process in textile industry. Conventionally pectin removal requires harsh chemicals and high temperature in most of the industrial processes. Thus sustainability and thermostability are prerequisites for any other alternative process. Pectinase also finds various applications in fruit juice industry, textile industry, paper and pulp industry, bioethanol production, improvement in antioxidant property of wine etc. The present review is an attempt to provide information about thermophilic pectinase and its application towards industrial sector.

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INTRODUCTION

Pectin is a heteropolysaccharide molecule composed of 1,4-linked galacturonate chains with a high percentage of methyl esterification (Fig. 1). It is a cementing material in primary cell wall and middle lamella of plants and known for its stability and integrity (Khan *et al*., 2013). Pectin is composed of as many as 17 different monosaccharides (Mollet *et al*., 2003). The basic structure of pectin consists of three major groups of polysaccharides, viz. (i)Homogalacturonan (HG), (ii) Rhamnogalacturonan-I (RG-I) and Rhamnogalacturonan-II (RG-II) containing majority of D-galacturonic acid along with xylogalacturonan (XGA), Apiogalacturonan (ApGA), Galacturonogalacturonan (GaGA), Galactogalacturonan (GGA), and Arabinogalacturonan (ArGA) to a lesser extent (Yapo, 2011).

Homogalacturonan (HG): It is the most abundant pectic polysaccharide. It is a linear chain of C-6 methyl esterified and C-2 or C-3 acetylated D-galacturonic acid. D-galacturonic acid units are linked together by α-1,4-glycosidic bond. It constitutes smooth region of pectin (Wolf *et al*., 2009).

Rhamnogalacturonan-I (RG I): Rhamnogalacturonans-I (RGs-I) are complex, heterogeneous and branched structural components of the primary cell wall of plants.

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RGs-I are made up of repeating diglycosyl [→2)α-L-Rhamnose (1→4) α-D-Galacturonic Acid(1→], which are branched at O-4/O-3 positions by 4 different side chain types, viz. (1→5)-α-L-arabinan, (1→4) β-D-galactan, arabinogalactan I and arabinogalactan-II (Yapo, 2011).

Rhamnogalacturonan-II (RG-II): RG-II has highly conserved structure as compared to HG and RG-I. It is a complex polysaccharide composed of α-1,4-linked homogalacturonan backbone having four different side chains. In plant cell wall, it exists as a dimer cross-linked by a borate di-ester. Pe´rez *et al*., (2003) reported twelve different glycosyl residues in RG-II including 3-deoxy-D-manno-octulosonic acid (Kdo) and the rare aceric acid (AceA), apiose (Api), and 3-deoxy-D-lyxo heptulosonic acid (Dha) (Voxeur *et al*., 2012). Pectin in spite of being important for plants also imposes numerous side effects in fruit juice, textile, and paper industry. These are turbidity, cloudiness and bitterness in the fruit juices; making the dyeing process of textile industry complex, imposing problem for bio fuel production etc. Therefore, pectinase can be used to rule out the above side effects of pectins (Hoondal *et al*., 2002). Pectinase is a general term which is used for a group of enzymes, such as pectin lyase, pectin methylesterase and polygalacturonase (Arunachalam and Asha, 2010). Plants pectinase play an important role in cell elongation, growth and fruit ripening while microbial pectinase are important in plant pathogenesis, symbiosis and recycling of nutrients by decomposition of plant deposits (Kumari *et al*., 2013).

Microbially derived pectinase appears more useful over plant and animal derived pectinase, because of cheap production, ease of genome manipulation, faster product recovery and freedom from harmful substances (Chaudhri and Suneetha, 2012). Further the social and political issues are also not linked with the use of microbial pectinase. Pectinase production shares about 10% of the overall manufacturing of enzyme preparations (Pedrolli *et al*., 2009). Microbial pectinase accounts for 25% of the global food enzymes sales (Murad *et al*., 2011). Pectinase are one of the foremost cosmopolitan enzymes distributed among bacteria (Vidhyasagar *et al.,* 2013), fungi (Adesina *et al*., 2013), yeast (Martos *et al.,* 2013), insects (Shen *et al.,* 2005), nematodes (Patil *et al*., 2012b) and plants (Rondan-Sanabria *et al*., 2006). Literature reveals that pectinase are widely studied for various applications, but there are very few reports about thermophilic pectinase, therefore the manuscript reviews the information available about thermophilic pectinase, their production and applications.

Pectinase: Sharma *et al*., (2013) demonstrated that complete degradation of pectin requires several pectinolytic enzymes. These enzymes can be broadly classified into following groups: -

- Pectin methyleatease, PME (3.1.1.11): It is the first enzyme (Pedrolli *et al*., 2009) of pectin degradation pathway catalyzing the hydrolysis of the methyl ester groups of pectin into pectic acid and methanol (Mckay, 1988).
- Pectin acetylesterase, PAE (3.1.1.6): Pectin acetylesterase catalyzes the hydrolysis of the acetyl ester groups of pectin molecule with the liberation of pectic acid and ethanol (Jayani *et al.,* 2005).
- Polygalacturonases, PG (EC. 3.2.1.15): It is a depolymerase catalyzing the hydrolysis of 1, 4-glycosidic linkages in linear homogalacturonan regions of pectic polymers (Sharma *et al*., 2013). These are of two types on the basis of their cleavage pattern: Exo-PG and endo PG. Exo-PG cleaves through terminal end of pectin chain whereas endo-PG acts randomly throughout the chain (Sharma *et al.,* 2012).
- Pectate lyase, PL (4.2.2.2): It catalyzes the transelimination cleavage of α-1, 4-glycosidic linkage of pectic acid in either sequential (Exo-PL) or random manner (Endo-PL) (Pedrolli *et al*., 2009).
- Pectin lyase, PNL (4.2.2.10)**:** It catalyzes the transelimination cleavage of α-1, 4- glycosidic linkage of pectin molecule in sequential manner resulting in the release of 4, 5-unsaturated oligogalacturonides (Yadav *et al*., 2009)**.**

In addition to above, other enzymes catalyzing the pectin degradation are:

- α–L–rhamnosidases, (EC. 3.2.1.40): It hydrolyzes rhamnogalacturonan in the pectic backbone.
- α–L–arabinofuranosidases, (EC. 3.2.1.55): It catalyzes L–arabinose side chains by adding water molecule.
- Endo–arabinase (EC. 3.2.1.99): This acts on arabinan side chains in pectin (Takao *et al.,* 2002).

Dhiman *et al*., (2013) classified pectinase into acidic and alkaline pectinase depending on their pH optima. Fungal pectin lyase showed maximum activity in acidic range of pH while bacterial counterpart was found more active in alkaline pH (Jayani *et al*., 2005). Table 1 shows various types of pectinase produced by microorganisms.

Mechanism of action

Pectin methylesterase: Johansson *et al*., (2002) proposed the mechanism for PME. Interestingly, the active site of PME lacks serine and histidine which are usually present in functionally related esterases (Jenkins *et al*., 2001). The active site of pectin methylesterase is lined by several conserved aromatic amino acid residues. Among these Asp136 and Asp157 is working as general acid and base to catalyse the ester bond. Initially, Asp157 acts as a nucleophilie for carboxy methyl carbonyl carbon atom while Asp136 acts as an acid and produce methanol from methylated α-1,4 D-galacturonosyl units. The active site is restored back to its original position in the next step when Asp136 extracts hydrogen from water molecule by breaking covalent bond between substrate and Asp136.

Polygalacturonases: These are inverting glycoside hydrolase which change the anomeric configuration of product during the reaction. They also follow the general acid- base catalysis mechanism. In the reaction a proton is donated by Asp173 to glycosidic oxygen. The catalytic base guides the nucleophilic attack of a water molecule on the anomeric carbon of galacturonate species at -1 subsite. The carboxyl group is required at +1 subsite for substrate binding (Armand *et al*., 2000; Pages *et al*., 2000).

Pectin lyase and Pectate lyase: In case of pectate lyase and pectin lyase, enzymatic cleavage occurs by β-transelimination mechanism and results in the formation of 4-5 unsaturated galacturonosyl residue (Petersen *et al*., 1997). Sharma *et al*., (2013) reviewed the biochemical structure of pectinase and proposed that in both lyases, β- elimination reaction occurs in three steps: (a) neutralization of the carboxyl group adjacent to the glycosidic bond, (b) removal of the C5 proton and (c) transfer of the proton to the glycosidic oxygen (Fig. 2).

Thermophilic enzymes: Thermophilic reactions are very important to chemical industry to solubilize sparingly soluble compounds and to lower the viscosity of environment. Another advantage of thermophilic enzyme based reaction is that they are less susceptible towards microbial contaminations (Dhiman *et al*., 2013). Thermophilic enzymes have various advantages in commercial applications. Their most attractive feature is their ability to tolerate high temperatures. In addition to above these enzymes are also found active in the presence of various denaturants e.g. guanidinium hydrochloride and urea (Kujo *et al*., 1998), detergents such as Triton X-100 and sodium dodecyl sulfate (Sako *et al*., 1997) and organic solvents (Turner *et al*., 2007). They also exhibit activity in broad range of pH (Kristjansson, 1989). Kumar *et al*., (2000) observed that thermostability of an enzyme depends upon various factors such as hydrophobicity (Dill, 1990), no. of ion pairs, structural rigidity and tendency of helix formation, presence of glycine, cysteine and analine and aromatic amino acids (Vieille and Zeikus, 2001) in it. In a study, Scandurra *et al*., (1998) performed various substitutions to increase the hydrophobicity in the core region of the protein molecule and found that mutant protein stable at high temperature.

Thermophilic Enzymes Production: Microorganisms are considered as a main source for thermophilic enzymes due to rapid growth rate and shorter life span. However, some xerophytes are also reported as source of thermophilic enzymes (Ravikumar *et al*., 2011). Thermostable pectinase have been reported from like *Clostridium stercorarium* (Zverlov *et al*., 2000), *Thermoascus aurantiacus* (Martins *et al*., 2002), *Sporotrichum thermophile* (Kaur *et al*., 2004), *Aspergillus fumigates* (Phutela *et al*., 2005), *Mycotypha* sp. strain No. AKM1801 (Venugopal *et al*., 2007), *Bacillus subtilis* SS (Ahlawat *et al*., 2008), *Penicillium canescens* I-85 and *Aspergillus niger T 1-1 (thermotolerant)* (Kutateladze *et al*., 2009), *Bacillus pumilus* dcsr1 (Sharma and Satyanarayana, 2012), Rhizomucor pusilis *(Siddiqui et al., 2012) and Bacillus halodurans* M29 (Mei *et al*., 2013) and others. *Aspergillus* was widely used fungi for the industrial production of thermophilic pectinolytic enzymes (Naidu and Panda, 1998), whereas many species of *Bacillus* were also used for the production of alkaline thermophilic pectinases (Dhiman *et al*., 2013).

Birgisson *et al*., (2004) produced a thermostable polygalacturonase from a mould *Sporotrichum thermophile*, which showed maximum activity at 55 °C. Soriano *et al*., (2006) cloned the gene *yvpA* from *Bacillus subtilis* and expressed in *Escherichia coli*. The enzyme was purified by His-tag affinity chromatography and characterized. The optimum temperature and pH were 65 °C and 10 respectively. The enzyme exhibited maximum activity on 22% esterified citrus pectin. Yuan *et al*., (2012) also cloned a pectate lyase gene from *Streptomyces* sp. S27 and expressed in *E.coli* Rostta (DE3). The pH and temperature optima was found to be 10.0 and 60 °C respectively and proved its candidacy for textile industry. Metagenomics is the genomic analysis of microorganisms by direct extraction and cloning of DNA from unculturable microorganisms from soil (Handelsman, 2004). It gives the complete genomic profile of proposed samples and helpful in novel gene discovery. Singh *et al*., (2012a) isolated a gene encoding thermostable pectinase from soil metagenome sample. The gene sequence corresponded to an open reading frame of 1,311 bp encoding a translation product of 47.9 kDa. It showed maximum (93 %) identity to a *Bacillus licheniformis* glycoside hydrolase. The temperature and pH optima of this protein was found 70 ^{0}C and 7.0 respectively. Pectinase production was also tried through solid state fermentation (SSF) technique. Pectinase production under SSF is not only economical but eco-friendly also (Mrudula and Anitharaj, 2011). Moreover, production through SSF also reduces the cost due to the efficient utilization of waste enabling a kind of value addition to it (Singh *et al*., 2012b). Kaur *et al*., (2004) found that the protein produced by SSF was stable over a wide range of temperature and pH. Besides above, the SSF produced enzyme had shown lesser susceptibility towards catabolic repression than pectinase produced by submerged fermentation (SmF). Various solid agricultural and agro–industrial residues have been used as substrates for the production of pectinase in SSF. These include soya bran (Castilho *et al*., 2000), cranberry and strawberry pomace (Zheng and Shetty, 2000), orange bagasse, sugar cane bagasse and wheat bran (Martins *et al*., 2002), orange peel (Mrudula and Anitharaj 2011), carrot waste (Patil *et al*., 2012a), pomegranate peel, citrus peel powder, spent tea leaves, sunflower leaf, cotton oilseed cake, mustard oilseed cake, sesame oilseed cake, wheat straw, wheat bran, sun hemp stalks, sunflower stalks, sunflower, sugarcane bagasse, ramie fibre, sun hemp fibre, rice straw and pineapple pulp (Sharma and Satyanarayana, 2012).

Among these, orange peel was considered as a good substrate and inducer for pectinase production as it contains higher amount of pectin (Mrudula and Anitharaj, 2011).

Applications: The first commercial application of pectinase was reported in 1930 (Farooqui *et al*., 2012; Pasha *et al*., 2013). Earlier, they were used for the preparation of wines and fruit juices. Thermostable pectinase finds major use in fruit processing, textile processing and in pulp and paper industry.

Fruit juice industry: The largest industrial application of pectinase is in fruit juice extraction and clarification process (Pasha *et al*., 2013). Pectinase addition in the extraction process improves the fruit juice yield by decreasing the juice viscosity and turbidity (Ghorai *et al*., 2009; Teixeira *et al*., 2011) and by decreasing the fermentation time (Piatka *et al.,* 2010). The treatment of fruit juices with pectinases was also accountable to increases in phenolic and antioxidant content of them (Sharma *et al*., 2013). Pedrolli *et al*., (2009) reviewed the applications of pectinase in fruit juice extraction and observed yield more than 90 % with a decrease in viscosity up to 62 % as compared to traditional method. Jayani *et al*., (2005) also suggested the use of pectinase to soften the peel of citrus fruits for pectin removal in industry. The extraction of banana juice is the most important step for banana syrup production. The pulpy and pectinous nature of banana forces the enzymatic liquefaction process to be carried out at high temperature. Thermostable pectinase here finds application as evidenced by Tadakittisarn *et al*., (2007). Similarly, Swain and Ray, (2010) also demonstrated the use of thermostable exopolygalacturonase of *B. subtilis* CM5 for carrot liquefaction. Piatka *et al*., (2010) and Joshi *et al*., (2011) also emphasized on the use of pectinase to enhance the juice yield with respect to moisture, total soluble solids, total sugars, acidity, lower crude fiber, vitamin C etc.

Textile industry: Cotton remains a universal fiber of choice among the world's increasing population. It is estimated that approximately 20 million tons of cotton is processed worldwide yearly (Menezes and Choudhari, 2011). There is, however severe environmental costs associated with the widespread use of cotton (Dhiman *et al*., 2013). The processing of cotton fiber in textile industry requires harsh chemicals such caustic soda, hydrogen peroxide etc. which cause serious environmental pollution. Rocky, (2012) reported that 75% of the organic pollutant level arising from textile finishing is derived from the preparation of cotton goods.

Therefore, it is meaningful to search for commercially viable, economical and eco-friendly alternative method/s over traditional methods. Enzymes provide various alternatives, environment and fiber friendly procedures by replacing or improving the existing traditional procedures. These enzymatic methods are based on the use of alkaline pectinase in conjugation with amylases, lipases, cellulases and other hemicellulolytic enzymes (Ahlawat *et al*., 2009; Preša and Tavčer 2008). Desizing is the process of removing the undesirable size material from the warp yarns in woven fabrics (Mojsov, 2012). The process must be carried out by treating the fabric with chemicals such as acids, alkali or oxidizing agents at high temperature. In addition to above, their use was also found to improve quality of fabric and safety aspects of textile workers also (Dalvi *et al*., 2007; Preša and Tavčer 2008).

Table 1. Pectinase produced by microorganism

S. No	Name Of Organism	Type Of enzyme	References
1	*Enterobacter aerogenes* NBO2	Polygalacturonase	Darah *et al*. (2013)
2	*Wickerhanomycesanomalus*	Polygalacturonase	Martos *et al*. (2013)
3	*Penicillium griseoroseum recombinant strains*	Polygalacturonase	Teixeira *et al*. (2013)
4	*Aspergillus flavus,*	Polygalacturonase	Deskmukh *et al*. (2012)
5	*Aspergillus niger*	Polygalacturonase	Deskmukh *et al*. (2012)
6	*Aspergillus oryzae*	Polygalacturonase	Deskmukh *et al*. (2012)
7	*Paecilomycesvariotii NFCCI 1769*	Polygalacturonase	Patil *et al*. (2012b)
8	*Rhizomucorpusilis*	Polygalacturonase	Siddiqui *et al*. (2012)
9	*Bacillus sphaericus* (MTCC 7542)	Polygalacturonase	Jayani *et al*., (2010)
10	*Botrytis cinerea*	Pectin Methyl esterase	Riegnault*et al*. (1994)
11	*Curvulariainaequalis*(Shear)	Pectin Methyl esterase	Afifi *et al*. (2002)
12	*Aspergillus japonicus*	Pectin Methyl esterase	Semenova*et al*. (2003)
13	*Erwinia chrysanthem*	Pectin Methyl esterase	Oskar and Stefan. (2004)
14	*Streptomyces sp.*	Pectin Methyl esterase	AD and Hussein. (2010)
15	*Aspergillus niger*	Pectin Methyl esterase	Huang *et al*., (2010)
16	*Thermoascusaurantiacus*	Pectin lyase	Martins *et al*. (2002)
17	*Penicillium viridicatum*	Pectin lyase	Silva D *et al*. (2002)
18	*Rizopusoryzae*	Pectin lyase	Hamdy. (2005)
19	*Penicillium canescens*	Pectin lyase	Sinitsyna*et al*. (2007)
20	*Penicillium oxalicum*	Pectin lyase	Yadav *et al*. (2007)
21	*Aspergillus flavus*	Pectin lyase	Yadav *et al* (2008)

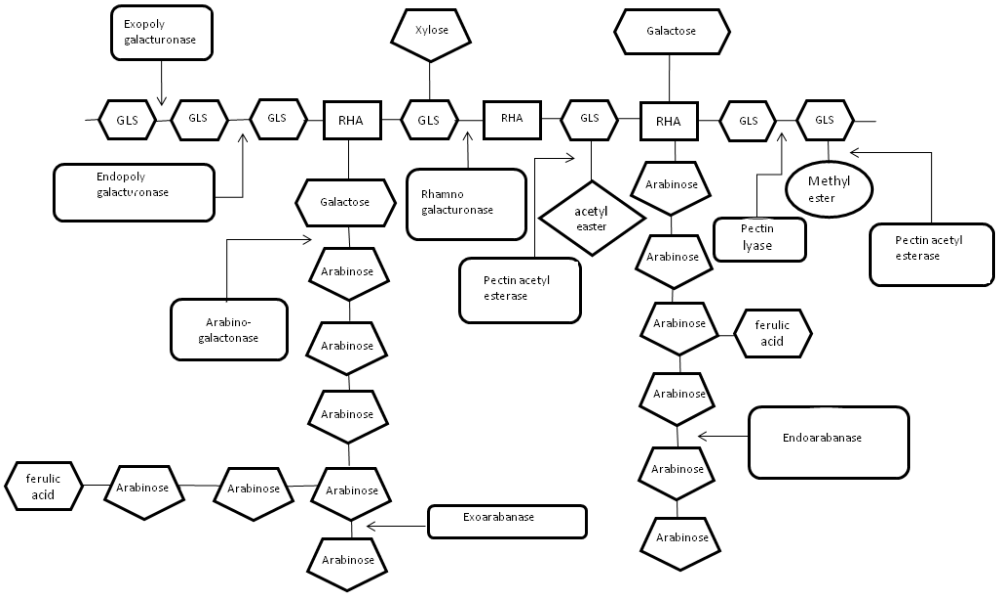

Figure 1. Activities of pectinases on pectin substrate. Abbreviations: GLS, galacturonic acid; RHA, rhamnose

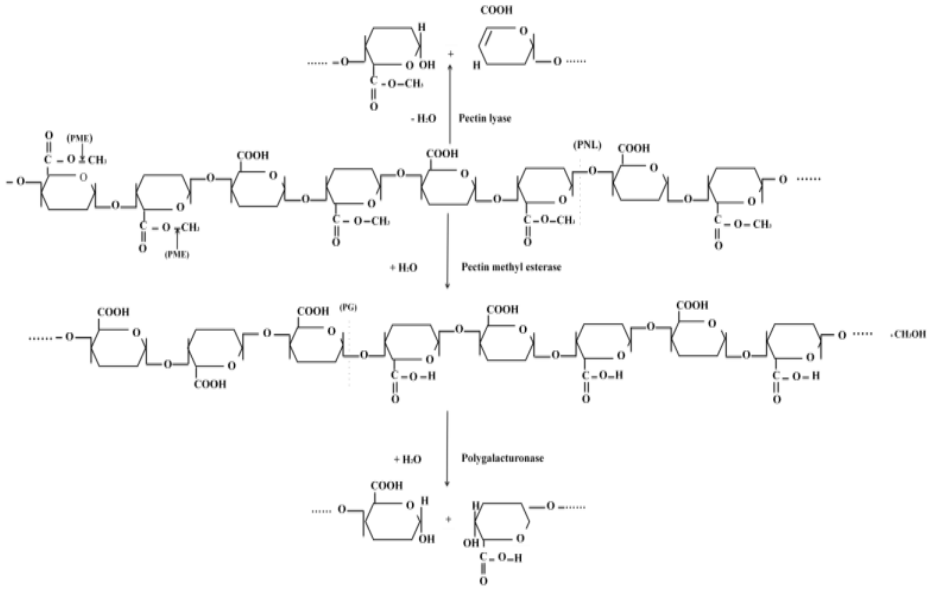

Figure 2.Degradation of a pectin chain by three type of pectinases

Biopreparation or Bioscouring is the process that targets non-cellulosic impurities of textile fabrics to make it hydrophilic and suitable for other wet processes (Mojsov, 2012). In conventional procedure, the removal of non-cellulosic impurities is done with caustic alkaline solution at high temperature to achieve uniform dyeing and finishing. This procedure requires huge amount of water for rinsing, high energy and yields the environment damaging waste effluent (Preša and Tavčer 2008; Rocky 2012). In recent years, research has been directed towards the discovery of environmental friendly enzymes that replace chemical alkaline scouring in textile industry (Rocky, 2012). The scouring with pectinase was observed with remarkable improvement in the absorbency and whiteness of the textile fabric (Karapinor and Sarisik, 2004). Thermostable pectinase of *B. subtilis* was used for bioscouring on desized cotton fabric and it resulted in enhancement in whiteness by 1.2 %, tensile strength by 1.6 % and tearness by 3.0 % as compared to traditional alkaline scouring (Ahlawat *et al*., 2009). Vigneshwaran *et al*., (2011) optimized bioscouring process variables (enzyme concentration, temperature and reaction time) and suggested that bioscouring of cotton fiber with pectinase should be done at 55- 60 ^{0}C for 45- 60 min with 5-6 % enzyme concentration for higher water absorbency and wax removal to get good yield. The whiteness of cotton fiber is of prime importance. The plant's natural pigments associated with lint were responsible for greyness thus has to be removed. Their removal imposes complication as starch and its derivatives (Sawhney *et al*., 2004) are used in their earlier steps of processing. Besides plant's pigments starch, plant's wax material, fats, proteins and pectins (Tzanova *et al*., 2001) were also involved to decrease the whiteness index of cotton fiber. In a study, Yachmenev *et al*., (2001) here showed the importance of alkaline pectinase and experimentally demonstrated its use to increase whiteness and absorbency. Pectinase also plays a significant role in degumming of bast fibers (*Grewia optiva*) to increase tensile strength and brightness. A thermostable polygalacturonase of *Bacillus sp* was utilized for degumming of sunn-hemp and ramie fibre (Kapoor *et al*., 2001; Kashyap *et al*., 2001)). In a study, Sharma and Satyanarayana, (2012) used the pectinase of *Bacillus pumilus* dcsr1 to increase tensile strength and brightness of cotton fiber. Guo *et al.,* (2013) also used pectinase of *Bacillus sp.* Y1 in conjugation with H_2O_2 and were able to improve the brightness of ramie fiber up to 83.7 %.

Paper and pulp industry: The use of chlorine containing bleaching compounds in paper and pulp industry results in generation of toxic, mutagenic and bioaccumulating organo-chlorine byproducts. These are responsible for serious nuisance in the ecosystem (Hebeish *et al*., 2013). The use of pectinase here finds application to avoid toxicity of chlorinated compounds (Ahlawat *et al*., 2007) in the ecosystem. A synergistic action of thermos table xylano-pectinolytic enzymes from *Bacillus pumilus* was also evaluated for the pre-bleaching of kraft pulp and their use resulted in 25% reduction in active chlorine consumption without any decrease in brightness. Physical parameters (Technical Association of Pulp and Paper Industry standard methods, 1996) such as Burst factor, tear factor, breaking strength and brightness were also improved after enzymatic treatment (Kaur *et al*., 2010). The industrial use of pectinase (Novozym 863) was also demonstrated by Reid and Richard, (2004) to decrease cationic demand of peroxide-bleached mechanical pulp. Moreover Ahlawat *et al*., (2008) also investigated the suitability of alkaline thermostable pectinase from *Bacillus subtilis* SS in paper and pulp industry and found an increase in brightness and whiteness with a reduction in chemical oxygen demand value of the industrial effluent.

Biomass utilization: The investigation for a fossil fuel alternative is of worldwide importance and research is focused to convert biomass into bio-ethanol as an alternative to fossil fuels is under the way. Biomass is the most important renewable source of energy in terms of technical and economic feasibility. Several pectin rich biomass such as cassava pulp (Sriroth *et al*., 2000; Apiwatanapiwat *et al*., 2013), apple pomace (Canteri-Schemin *et al*., 2005), citrus waste (Lopez *et al.,* 2010) and sugar beet pulp (Rorick *et al*., 2011) was used for the production of bioethnol. Cassava pulp is considered as good source of starch but pectin network interfere in its extraction. Here Sriroth *et al*., (2000) demonstrated high efficiency of starch extraction from cassava pulp by using cellulase and pectinase. The presence of pectin also influences the production of fermentable sugar syrup for sugar beet plant (Fernandes *et al*., 2008). The traditional bioethanol production (hydrolysis of cellulose and starch rich biomass followed by ethanolic fermentation by yeast) is not feasible in case of pectin rich biomass owing to non-fermentable galacturonic acid and arabinose. Doran *et al*., (2000) and Edwards *et al*., (2011) engineered *E. coli* for simultaneously production of cellulase and pectinase activity and obtained more yield in terms of ethanol production by fermenting cellulose and starch.

Prebiotics/Functional Foods: A prebiotic is defined as "a selectively fermented substance that allows specific changes in the composition and/or activity of the gut microbiome to benefit host immune system (Gullón *et al*., 2011). In recent years, novel applications of pectinase were noticed as prebiotic component or functional food (Joshi *et al*., 2013). In new generation prebiotics, pectin and pectin derived oligosaccharides (PDO) are emerging as an excellent candidate. It has been reported that intestinal bacteria ferment more rapidly demethylated pectin to produce short-chain fatty acids (SCFA) *viz*. health promoting acetate, propionate, and butyrate (Manderson *et al.,* 2005; Gullón *et al*., 2011). In addition to this, Salazar *et al*., (2009) recognized a significant increase in bifidobacteria, lactobacillus, *Eubacterium rectale* numbers (Olano- Martin *et al*., 2003; Manderson *et al*., 2005), SCFA (particularly acetate, propionate, and butyrate), lactate and their beneficial effect on host's health in the presence of PDO. Jackson *et al*., (2007) demonstrated that oral feeding with galactuose containing citrus pectin (heat treated) inhibit spontaneous prostate carcinoma metastasis by competing with natural galectins and also induce apoptosis in cancer cells. It was also believed that pectin increases the viscosity in the intestinal tract, excretion of fecal bile acids as well as neutral sterol which results in reduced cholesterol level (Khan *et al*., 2013). Pectin and PDO, apart from protecting the host against bowel inflammatory diseases were also found involved in regulating the release of gut hormones (Tolhurst *et al*., 2012).

Improvement in antioxidant property of fruit juices: Fruits and vegetables contain bioactive secondary metabolites like, e.g., polyphenol, anthocynins and various amounts of dietary fibers. During the last few years, many cases were studied to recognize the role of pectinase in improvement of phenolics and antioxidant content of juices and its potential benefits for human health. Oszmiański *et al*., (2011) applied two commercially available pectinase; Pectinex XXL and Pectinex

Ultra SPL in apple juice processing and found higher phenolics contents (1520 mg/L) as compared to untreated juice (441 mg/L). Besides above, pectinase was also used to decrease the astringency in fruit juices by solubilizing anthocyanins without leaching out procyanadin polyphenols (Farooqui 2012). Armada *et al*., (2010) demonstrated the use of pectinase to improve stability, taste and structure of red wines. The wines produced by using pectinase were found higher in yield, anthocyanin level, total phenolics, tannins, clarity as well as colour intensity (Sharma *et al*., 2013).

Conclusion

Temperature stability is an important characteristic of a biocatalyst for use in industrial applications. The thermophilic enzymes are seeking attention in research because it is a difficult and expensive to control temperature during the large scale fermentation processes. Concomitantly, information obtained from pectinase genome sequence have opened up exciting new possibilities for biotechnological opportunities based on extreme thermophiles that go beyond single-step biotransformation. Metagenomics and enzyme engineering approaches for exploring the novel opportunities of thermophilic pectinase with desire characteristics can be appreciated.

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