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Review

Microbial pectinolytic enzymes: A review

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Abstract

Pectinases or petinolytic enzymes, hydrolyze pectic substances. They have a share of 25% in the global sales of food enzymes. Pectinases are one of the most widely distributed enzymes in bacteria, fungi and plants. Protopectinases, polygalacturonases, lyases and pectin esterases are among the extensively studied pectinolytic enzymes. Protopectinases catalyze the solubilization of 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. Pectinesterases liberate pectins and methanol by de-esterifying the methyl ester linkages of the pectin backbone. Pectinolytic enzymes are of significant importance in the current biotechnological era with their all-embracing applications in fruit juice extraction and its clarification, scouring of cotton, degumming of plant fibers, waste water treatment, vegetable oil extraction, tea and coffee fermentations, bleaching of paper, in poultry feed additives and in the alcoholic beverages and food industries. The present review mainly contemplates on the types and structure of pectic substances, the classification of pectinolytic enzymes, their assay methods, physicochemical and biological properties and a bird's eye view of their industrial applications.

Keywords: Pectic substances; Pectinolytic enzymes; Protopectinases; Polygalacturonases; Lyases; Pectinesterases

Contents

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1. Introduction

In nature, microorganisms have been endowed with vast potentials. They produce an array of enzymes, which have been exploited commercially over the years. Pectinases are of great significance with tremendous potential to offer to industry [1]. They are one of the upcoming enzymes of the commercial sector, especially the juice and food industry [2] and in the paper and pulp industry [3,4].

Pectinolytic enzymes or pectinases are a heterogeneous group of related enzymes that hydrolyze the pectic substances, present mostly in plants. Pectinolytic enzymes are widely distributed in higher plants and microorganisms [5]. They are of prime importance for plants as they help in cell wall extension [6] and softening of some plant tissues during maturation and storage [7,8]. They also aid in maintaining ecological balance by causing decomposition and recycling of waste plant materials. Plant pathogenicity and spoilage of fruits and vegetables by rotting are some other major manifestations of pectinolytic enzymes [5,9–14].

It has been reported that microbial pectinases account for 25% of the global food enzymes sales. Almost all the commercial preparations of pectinases are produced from fungal sources [14]. *Aspergillus niger* is the most commonly used fungal species for industrial production of pectinolytic enzymes [15–17].

This review mainly concentrates on the types of pectinolytic enzymes, their classification, mode of action and the substrates on which they act. It also provides a bird's eye view of the possible applications of these enzymes in industrial sector.

2. The substrate

2.1. Pectic substances

Pectic substance is the generic name used for the compounds that are acted upon by the pectinolytic enzymes. They are high molecular weight, negatively charged, acidic, complex glycosidic macromolecules (polysaccharides) that are present in the plant kingdom. They are present as the major components of middle lamella between the cells in the form of calcium pectate and magnesium pectate [18]. That the middle lamella is largely composed of pectic substances, has been confirmed by the comparable uptake of ruthenium red by known pectic substances [19] and from the estimation of pectin by the use of alkaline hydroxylamine [20–22]. Pectic substances account for 0.5–4.0% of the fresh weight of plant material [2,23] (Table 1).

Contrary to the proteins, lipids and nucleic acids, being polysaccharides, pectic substances do not have a defined

Table 1
Composition of pectin in different fruits and vegetables

Fruit/vegetable	Tissue	Pectic substance (%)
Apple	Fresh	0.5–1.6
Banana	Fresh	0.7–1.2
Peaches	Fresh	0.1–0.9
Strawberries	Fresh	0.6–0.7
Cherries	Fresh	0.2–0.5
Peas	Fresh	0.9–1.4
Carrots	Dry matter	6.9–18.6
Orange pulp	Dry matter	12.4–28.0
Potatoes	Dry matter	1.8–3.3
Tomatoes	Dry matter	2.4–4.6
Sugar beet pulp	Dry matter	10.0–30.0

Table 2
Molecular weights of some pectic substances [23]

Source	Molecular weight (kDa)
Apple and lemon	200–360
Pear and prune	25–35
Orange	40–50
Sugar beet pulp	40–50

molecular weight. The relative molecular masses of pectic substances range from 25 to 360 kDa (Table 2).

2.2. Structure, classification and nomenclature of pectic substances

Pectic substances mainly consist of galacturonans and rhamnogalacturonans in which the C-6 carbon of galactate is oxidized to a carboxyl group, the arabinans and the arabinogalactans [5]. These substances are a group of complex colloidal polymeric materials, composed largely of a backbone of anhydrogalacturonic acid units [24,25]. The carboxyl groups of galacturonic acid are partially esterified by methyl groups and partially or completely neutralized by sodium, potassium or ammonium ions [2]. Some of the hydroxyl groups on C_2 and C_3 may be acetylated [26]. The primary chain consists of α-D-galacturonate units linked α-(1 → 4), with 2–4% of L-rhamnose units linked β-(1 → 2) and β-(1 → 4) to the galacturonate units [5] (Fig. 1). The rhamnogalacturonans are negatively charged at pH ≥ 5. The side chains of arabinan, galactan, arabinogalactan, xylose or fucose are connected to the main chain through their C_1 and C_2 atoms [27–29]. The above description indicates that the pectic substances are present in various forms in plant cells and this is the probable reason for the existence of various forms of pectinolytic enzymes.

The American Chemical Society classified pectic substances into four main types as follows [26]:

(I) *Protopectin*: is the water insoluble pectic substance present in intact tissue. Protopectin on restricted hydrolysis yields pectin or pectic acids.
(II) *Pectic acid*: is the soluble polymer of galacturonans that contains negligible amount of methoxyl groups. Normal or acid salts of pectic acid are called pectates.
(III) *Pectinic acids*: is the polygalacturonan chain that contains >0 and <75% methylated galacturonate units. Normal or acid salts of pectinic acid are referred to as pectinates.
(IV) *Pectin* (*Polymethyl galacturonate*): is the polymeric material in which, at least, 75% of the carboxyl groups of the galacturonate units are esterified with methanol. It confers rigidity on cell wall when it is bound to cellulose in the cell wall.

3. The pectinolytic enzymes

The pectinolytic enzymes may be divided in three broader groups as follows [7,10]:

(I) *Protopectinases*: degrade the insoluble protopectin and give rise to highly polymerized soluble pectin.
(II) *Esterases*: catalyze the de-esterification of pectin by the removal of methoxy esters.
(III) *Depolymerases*: catalyze the hydrolytic cleavage of the α-(1 → 4)-glycosidic bonds in the D-galacturonic acid moieties of the pectic substances.

Depolymerases act on pectic substances by two different mechanisms, hydrolysis, in which they catalyze the hydrolytic cleavage with the introduction of water across the oxygen bridge and trans-elimination lysis, in which they break the glycosidic bond by a trans-elimination reaction without any participation of water molecule (Fig. 2) [25,30]. Depolymerases can be subdivided into four different categories, depending on the preference of enzyme for the substrate, the mechanism of cleavage and the splitting of the glycosidic bonds [31]. Polygalacturonase and polymethylgalacturonase breakdown pectate and pectin, respectively by the mechanism of hydrolysis. However, polygalacturonate lyase and polymethylgalacturonate lyase breakdown pectate and pectin by β elimination, respectively. Depending upon the pattern of action, i.e. random or terminal, these enzymes are termed as Endo or Exo enzymes, respectively.

During the course of investigations and research conducted in recent years, the above classification of pectinolytic enzymes has slowly become obsolete. A more recent and elaborated classification of these enzymes has been provided in Table 3 [2,5,6,31].

3.1. Protopectinases

The enzyme that catalyzes the solubilization of protopectin was originally named protopectinase by Briton et al. [32]. Pectinosinase is also synonymous with protopectinase (PPase). Protopectinase catalyzes the following reaction:

$$\underset{\text{(insoluble)}}{\text{protopectin}} + H_2O \xrightarrow{\text{PPase}} \underset{\text{(soluble)}}{\text{pectin}}$$

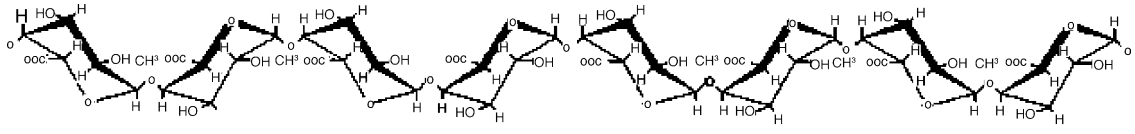

Fig. 1. Primary structure of pectic substances [31].

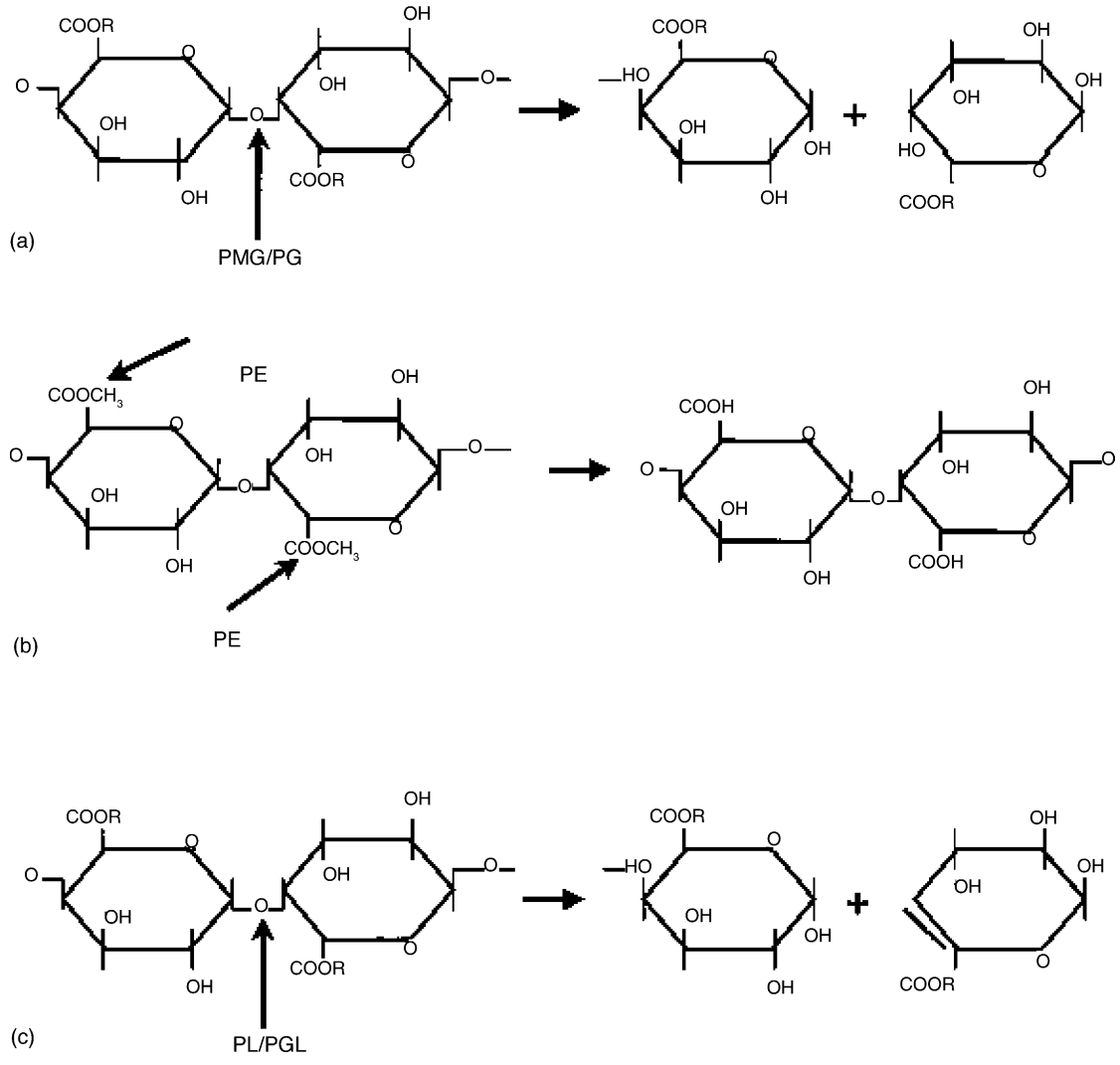

Fig. 2. Mode of action of pectinases: (a) R = H for PG and CH_3 for PMG; (b) PE; and (c) R = H for PGL and CH_3 for PL. The arrow indicates the place where the pectinase reacts with the pectic substances. PMG, polymethylgalacturonases; PG, polygalacturonases (EC 3.2.1.15); PE, pectinesterase (EC 3.1.1.11); PL, pectin lyase (EC-4.2.2.10) [28].

3.1.1. Classification

PPases are classified into two types, on the basis of their reaction mechanism [33–36]. A-type PPases react with the inner site, i.e. the polygalacturonic acid region of protopectin, whereas B-type PPases react on the outer site, i.e. on the polysaccharide chains that may connect the polygalacturonic acid chain and cell wall constituents.

3.1.2. Occurrence

A-type PPase are found in the culture filtrates of yeast and yeast-like fungi. They have been isolated from *Kluyveromyces fragilis* IFO 0288, *Galactomyces reesei* L. and *Trichosporon penicillatum* SNO 3 and are referred to as PPase-F, -L and -S, respectively [5]. B-type PPases have been reported in *Bacillus subtilis* IFO 12113 [37], *B. subtilis* IFO 3134 [38] and *Trametes* sp. [23], and are referred to as PPase-B, -C and -T, respectively. B-type PPases have also been found in the culture filtrate of a wide range of *Bacillus* sp. [7].

3.1.3. Assay methods

PPase activity is assayed by measuring the amount of pectic substance liberated from protopectin by the carbazole–sulphuric acid method [39]. The pectin concentration is measured as D-galacturonic acid from its standard curve. One unit of PPase activity is defined as the enzyme that liberates pectic substance corresponding to 1 μmol of D-galacturonic acid per millilitre of reaction mixture under assay conditions.

3.1.4. Physicochemical and biological properties

All three A-type PPases are similar in biological properties and have similar molecular weight of 30 kDa. PPase-F is an acidic protein, and PPase-L and -S are basic proteins. The enzymes have pectin-releasing effects on protopectin from various origins. The enzymes catalyze the hydrolysis of polygalacturonic acid; they decrease the viscosity, slightly increasing the reducing value of the

Table 3
An extensive classification of pectinolytic enzymes

Enzyme	E.C. no.	Modified EC systematic name	Action mechanism	Action pattern	Primary substrate	Product
Esterase						
1. Pectin methyl esterase	3.1.1.11		Hydrolysis	Random	Pectin	Pectic acid + methanol
Depolymerizing enzymes						
a. Hydrolases						
1. Protopectinases			Hydrolysis	Random	Protopectin	Pectin
2. Endopolygalacturonase	3.2.1.15	Poly-(1-4)-α-D-galactosiduronate glycanohydrolase	Hydrolysis	Random	Pectic acid	Oligogalacturonates
3. Exopolygalacturonase	3.2.1.67	Poly-(1-4)-α-D-galactosiduronate glycanohydrolase	Hydrolysis	Terminal	Pectic acid	Monogalacturonates
4. Exopolygalacturonan-digalacturono hydrolase	3.2.1.82	Poly-(1-4)-α-D-galactosiduronate digalacturonohydrolase	Hydrolysis	Penultimate bonds	Pectic acid	Digalacturonates
5. Oligogalacturonate hydrolase			Hydrolysis	Terminal	Trigalacturonate	Monogalacturonates
6. Δ4:5 Unsaturated oligogalacturonate hydrolases			Hydrolysis	Terminal	Δ4:5(Galacturonate)$_n$	Unsaturated monogalacturonates & saturated (n-1)
7. Endopolymethyl-galacturonases			Hydrolysis	Random	Highly esterified pectin	Oligomethylgalacturonates
8. Endopolymethyl-galacturonases			Hydrolysis	Terminal	Highly esterified pectin	Oligogalacturonates
b. Lyases						
1. Endopolygalacturonase lyase	4.2.2.2	Poly-(1-4)-α-D-galactosiduronate lyase	Trans-elimination	Random	Pectic acid	Unsaturated oligogalacturonates
2. Exopolygalacturonase lyase	4.2.2.9	Poly-(1-4)-α-D-galactosiduronate exolyase	Trans-elimination	Penultimate bond	Pectic acid	Unsaturated digalacturonates
3. Oligo-D-galactosiduronate lyase	4.2.2.6	Oligo-D-galactosiduronate lyase	Trans-elimination	Terminal	Unsaturated digalacturonates	Unsaturated monogalacturonates
4. Endopolymethyl-D-galactosiduronate lyase	4.2.2.10	Poly(methyl galactosiduronate) lyase	Trans-elimination	Random	Unsaturated poly-(methyl-D-digalacturonates)	Unsaturated methyloligogalacturonates
5. Exopolymethyl-D-galactosiduronate lyase			Trans-elimination	Terminal	Unsaturated poly-(methyl-D-digalacturonates)	Unsaturated methylmonogalacturonates

reaction medium containing polygalacturonic acid [40]. PPase-B, -C and -T have molecular weights of 45, 30, and 55 kDa, respectively. PPase-B and -C have an isoelectric point (pI) of around 9.0 whereas PPase-T has a pI of 8.1 [7]. PPase-B, -C and -T act on protopectin from various citrus fruit peels and other plant tissues, releasing pectin [7].

3.2. Polygalacturonases

Polygalacturonases (PGases) are the pectinolytic enzymes that catalyze the hydrolytic cleavage of the polygalacturonic acid chain with the introduction of water across the oxygen bridge. They are the most extensively studied among the family of pectinolytic enzymes.

3.2.1. Classification

The PGases involved in the hydrolysis of pectic substances are endo-PGase (E.C. 3.2.1.15) and exo-PGase (E.C. 3.2.1.67) (Table 3). PGases have the biological, functional and technical applications in food processing and plant–fungal interactions.

3.2.2. Occurrence

Endo-PGases are widely distributed among fungi, bacteria and many yeasts [41]. They are also found in higher plants and some plant parasitic nematodes [23]. They have been reported in many microorganisms, including *Aureobasidium pullulans* [42], *Rhizoctonia solani* Kuhn [43], *Fusarium moniliforme* [44], *Neurospora crassa* [45], *Rhizopus stolonifer* [46], *Aspergillus* sp. [47], *Thermomyces lanuginosus* [48], *Peacilomyces clavisporus* [49]. Endo-PGases have also been cloned and genetically studied in a large number of microbial species [50–57].

In contrast, exo-PGases occur less frequently. They have been reported in *Erwinia carotovora* [10], *Agrobacterium tumefaciens* [58], *Bacteroides thetaiotamicron* [59], *E. chrysanthemi* [60], *Alternaria mali* [61], *Fusarium oxysporum* [62], *Ralstonia solanacearum* [63], *Bacillus* sp. [60]. Exo-PGases can be distinguished into two types: fungal exo-PGases, which produce monogalacturonic acid as the main end product; and the bacterial exo-PGases, which produce digalacturonic acid as the main end product [23]. Occurrence of PGases in plants has also been reported [64–68].

3.2.3. Assay methods

PGase activity is determined on the basis of measuring, during the course of the reaction: (a) the rate of increase in number of reducing groups; and (b) the decrease in viscosity of the substrate solution [31].

The amount of reducing sugar can be readily measured by colorimetric methods like 3,5-dinitrosalicylate reagent method [69] and the arsenomolybdate–copper reagent method [70–73]. One unit of enzyme activity is defined as the enzyme that releases 1 μmol ml^{-1} min^{-1} galacturonic acid under standard assay conditions.

Viscosity reduction measurements have also found widespread use in determining the PGase activity [74]:

$$\%\text{reduction in viscosity} = \frac{T_o - T_t}{T_o - T_w}$$

where T_o, T_t and T_w represent the flow rate (in a capillary viscometer) in seconds for the reaction mixture without enzyme, the test mixture and water, respectively. The unit of enzyme activity is mostly selected as the amount of enzyme required for attaining a certain decrease of viscosity per unit time. However, this method has met with limited success. There is no direct correlation between viscosity reduction and number of glycosidic bonds hydrolyzed.

PGase activity can also be determined by the cup–plate method [75]. Cups are cut out from the solidified agar containing the substrate and are filled with the enzyme solution. After lapse of a certain period of time, the zones of degraded substrate are stained with iodine and quantified.

3.2.4. Physicochemical and biological properties

PGases isolated from different microbial sources differ markedly from each other with respect to their physicochemical and biological properties and their mode of action. Table 4 summarizes the biochemical and physicochemical properties of PGases obtained from various sources. Among the PGases obtained from different microbial sources, most have the optimal pH range of 3.5–5.5 and optimal temperature range of 30–50 °C. Two endo-PGases (PG I and PG II), isolated from *Aspergillus niger* have optimal pH range of 3.8–4.3 and 3.0–4.6, respectively [76]. Most of the PGase reported, work efficiently in acidic pH range but a few alkaline PGases have been also reported from *Bacillus licheniformis* [14] and *Fusarium oxysporum* f. sp. *lycopersci* [77] with optimum pH of 11.0. Barnby *et al.* [16] reported four isoenzymes, viz., PG I, PG II, PG III and PG IV with same molecular weight but differing in their isoelectric points. Although most of the PGases work in a temperature range of 30–40 °C, but a few PGases, which can catalyze the hydrolysis of pectic substances at higher temperatures have also been isolated from *Bacillus licheniformis* [14], *Bacillus* sp. KSM-P443 [60] and *Fusarium oxysporum* f. sp. *lycopersci* [77].

3.3. Lyases

Lyases (or transeliminases) perform non-hydrolytic breakdown of pectates or pectinates, characterized by a trans-eliminative split of the pectic polymer [23]. The lyases break the glycosidic linkages at C-4 and simultaneously eliminate H from C-5, producing a Δ 4:5 unsaturated product [25,30].

3.3.1. Classification

Lyases can be classified into following types on the basis of the pattern of action and the substrate acted upon by them (Table 3):

Table 4
Biochemical and physicochemical properties of some polygalacturonases

Source of PGase	Nature	Molecular weight (kDa)	pI	Specific activity (U mg^{-1})	K_m	Optimum temperature (°C)	Optimum pH	Temperature stability	pH stability	Reference
Aspergillus japonicus	Endo	38 (PG I)	5.6	–	–	30	4.0–5.5	–	–	[117]
	Endo	65 (PG II)	3.3	–	–	30	4.0–5.5	–	–	
Mucor flavus	–	40	8.3	–	–	45	3.5–5.5	40	2.5–6.0	[82]
Thermococcus aurantiacus	Endo	35	5.9	5890	0.13	55	5.0	60	4.0-6.5	[89]
Aspergillus niger	Endo	61 (PG I)	–	982	0.12	43	3.8–4.3	50	–	[76]
	Endo	38 (PG II)	–	3750	0.72	45	3.0–4.6	51	–	
Bacillus sp KSM-P410	Exo	45	5.8	54	1.3	60	7.0	50	7.0–12.0	[60]
Penicillium frequentans	Exo	63	–	2571	1.6	50	5.0	–	–	[84]
	Exo	79	–	185	0.059	50	5.8	–	–	
Aspergillus awamori	Endo	41	6.1	487	–	40	5.0	50	4.0–6.0	[47]
Yersinia enterocolitica	Exo	63	6.6	–	–	–	–	–	–	[90]
Bacillus licheniformis	Exo	38	–	209	–	69	11.0	–	7.0–11.0	[14]
Sclerotinia sclerotiorum	Endo	42 (PG8b)	4.8	–	0.8	50	4.0	–	–	[86]
	Endo	41.5 (PG8u)	4.8	–	0.5	50	3.5	–	–	
Stereum purpureum	Endo	42 (PG1a)	8.5	488	–	–	5.0	75	–	[87]
	Endo	44 (PG1b)	8.5	416	–	–	5.0	75	–	
	Endo	46(PG1c)	8.5	457	–	–	5.0	75	–	
Sclerotinia borealis	Endo	40	7.5	2088	-	40–50	5.0	50	3.5–5.5	[88]
Saccharomyces pastorianus	–	43	5.4	0.62	–	50	4.2	–	–	[89]
Saccharomyces cerevisiae IMI-8b	–	43	–	–	–	45	4.5	–	–	[27]
Fusarium oxysporum f. sp. *lycopersci*	Exo	38	–	209	–	69	11.0	–	7.0–11.0	[77]
Saccharomyces cerevisiae	Endo	39	–	2870	4.7	45	5.5	50	4.5–6.0	[133]
Kluyveromyces marxianus	Endo	496 (PG I)	6.3	102.6	–	–	–	–	–	[16]
	Endo	496 (PG II)	6.0	102	–	–	–	–	–	
	Endo	496 (PG III)	6.3	107.8	–	–	–	–	–	
	Endo	496 (PG IV)	5.7	97.6	–	–	–	–	–	

(I) endopolygalacturonate lyase (EndoPGL, E.C. 4.2.2.2);
(II) exopolygalacturonate lyase (ExoPGL, E.C. 4.2.2.9);
(III) endopolymethylgalacturonate lyase (EndoPMGL, E.C. 4.2.2.10);
(IV) exopolymethylgalacturonate lyase (ExoPMGL).

The reactions catalyzed by lyases can be illustrated as follows:

$$\text{polygalacturonate} \xrightarrow{\text{PGL}} \Delta 4:5 \text{ unsaturated galacturonates}$$

$$\text{polymethylgalacturonate} \xrightarrow{\text{PMGL}} \text{unsaturated methyloligogalacturonates}$$

3.3.2. Occurrence

Polygalacturonate lyases (Pectate lyases or PGLs) are produced by many bacteria and some pathogenic fungi with endo-PGLs being more abundant than exo-PGLs. PGLs have been isolated from bacteria and fungi associated with food spoilage and soft rot. They have been reported in *Colletotrichum lindemuthionum* [78], *Bacteroides thetaiotaomicron* [79], *Erwinia carotovora* [80], *Amucala* sp. [81], *Pseudomonas syringae* pv. *Glycinea* [82], *Colletotrichum magna* [83], *E. chrysanthemi* [84,85], *Bacillus* sp. [86–89], *Bacillus* sp. DT-7 [90], *C. gloeosporioides* [91,92].

Very few reports on the production of polymethylgalacturonate lyases (pectin lyases or PMGLs) have been reported in literature. They have been reported to be produced by *Aspergillus japonicus* [93], *Penicillium paxilli* [94,95], *Penicillium* sp. [96,97,98], *Pythium splendens* [99] *Pichia pinus* [100], *Aspergillus* sp. [101], *Thermoascus auratniacus* [102].

3.3.3. Assay methods

The most convenient method of following the activity of lyases is to measure the increase in absorbance at 235 nm due to formation of the Δ 4:5 double bonds produced at the non-reducing ends of the unsaturated products [5,103,104]. The molar extinction coefficients for PGL and PMGL are 4.6×10^3 and $5.5 \times 10^3\ M^{-1}\ cm^{-1}$, respectively. One unit of enzyme activity is defined as the amount of enzyme that releases 1 μmol of unsaturated product per minute under assay conditions. Reducing group methods [69–73] are also useful in determining the lyase activity. Viscosity reduction method [74], in conjunction with a reducing group method

or along with intermediate product analysis by HPLC or GC, can be used to distinguish between endo- and exo-splitting enzymes [104].

3.3.4. Physicochemical and biological properties

Most of the pectin lyases have been reported from microorganisms and there are scanty reports of their presence in plants and animals [5]. In bacteria, lyases are the largest group of pectinolytic enzymes and are directly involved in plant pathogenicity [105]. PGLs have an absolute requirement for Ca^{2+} ions [82] and hence chelating agents such as EDTA act as their inhibitors whereas PMGLs do not have an absolute requirement of cations but are stimulated by Ca^{2+} and other cations [5]. Interestingly, endo-PMGL is the only enzyme known to be able to cleave, without the prior action of other enzymes, the α-1,4-glycosidic bonds of highly esterified pectins [23,97].

Properties of some of the lyases have been shown in Table 5. Most of the lyases have molecular weights ranging between 30 and 40 kDa, with isoelectric point ranging from 7.0 to 11.0. They have pH optima in the alkaline range (7.5–10.0) and temperature optima of 40–50 °C. Two PMGLs, L_1 and L_2, from *Aureobasidium pullulans* LV-10, showed optimal activity at pH 5.0 and 7.5, respectively and at 40 °C [106]. Singh et al. [87] reported a thermostable exo-PGL from *Bacillus* sp., which showed maximum activity at pH 11.0 and 69 °C and was dependent on Ca^{2+} for its activity. Thermostable lyases have also been reported from *Bacillus* sp. TS 47, *Thermoascus auratniacus* [102] and *Fusarium monoliforme* [105]. Immobilized lyase from *Aspergillus japonicus* had a K_m value of 0.16 and was best active at pH 6.0 and 55 °C [107]. Many purification studies have also been carried out on lyases, which led to increase in specific activities of the enzymes with significant recoveries [81,108].

3.4. Pectinesterase

Pectinesterase (PE, Pectin pectlylhydrolase, E.C. 3.1.1.11), often referred to as pectinmethylesterase, pectase, pectin methoxylase, pectin demethoxylase and pectolipase, is a carboxylic acid esterase and belongs to the hydrolase group of enzymes [109]. It catalyzes the deesterification of methyl ester linkages of galacturonan backbone of pectic substances to release acidic pectins and methanol [110]. The resulting pectin is then acted upon by polygalacturonases and lyases [23,111]. The mode of action of PE varies according to its origin [112]. Fungal PEs act by a multi-chain mechanism, removing the methyl groups at random. In contrast, plant PEs tend to act either at the non-reducing end or next to a free carboxyl group, and proceed along the molecule by a single chain mechanism. The reaction catalyzed by PE can be represented as follows [113]:

$$\text{pectin} + n\text{H}_2\text{O} \xrightarrow{\text{PE}} \text{pectate} + n\text{C}_2\text{H}_5\text{OH}$$

3.4.1. Occurrence

PE activity is implicated in cell wall metabolism including cell growth, fruit ripening, abscission, senescence and pathogenesis [114,115]. Commercially PE can be used for protecting and improving the texture and firmness of

Table 5
Biochemical and physicochemical properties of some lyases

Source of lyase	Nature	Molecular weight (kDa)	pI	K_m	Optimum temperature (°C)	Optimum pH	Temperature stability (°C)	pH stability	References
Erwinia carotovora	PGL (PL I)	36–38	10.7	0.12	50	10.0	–		[116]
	PGL (PLII)	36–38	10.1	1.1	60	10.0	–		
Bacteroides thetaiotaomicron	PGL	74	7.5	0.04–0.07	–	8.7	–		[59,79]
Aureobasidium pullulans LV-10	PMGL (L1)	89	–	–	40	5.0	50	3.5	[106]
	PMGL (L2)	55	–	–	40	7.5	40	5.9	
Penicillium italicum	PMGL	22	8.6	3.2	40	6.0–7.0	50	8.0	[97]
Bacillus macerans	PGL	35	10.3	–	60	9.0	–		[108]
Aspergillus japonicus	PMGL	–	7.7	0.16	55	6.0	–		[107]
Penicillium adametzii	PMGL	–	–	–	60	8.0	40	7.0	[98]
P. citrinum	PMGL	–	–	–	45	7.0	40	7.0	
P. janthinellum	PMGL	–	–	–	40	6.5	40	7.0	
Amycolata sp.	PGL	31	10.0	0.019	70	10.3	50	6.0–8.0	[81]
Pythium splendens	PMGL	23	8.0	–	–	8.0	50		[99]
Bacillus sp.	PGL	38	–	–	69	11.0	60	7.0–11.0	[87]
Yersinia enterocolitica	PGL	55	5.2	–	–	–	–		[90]
Pichia pinus	PMGL	90	–	0.33	–	4.5	50		[100]
Bacillus sp. TS44	PGL	50	5.3	–	70	8.0	70	11.0	[88]
Thermoascus auratniacus	PMGL	–	–	–	65	10.5–11.0	70	4.0	[89]
Fusarium monoliforme	PGL	–	–	–	–	8.5	75		[105]

several processed fruits and vegetables as well as in the extraction and clarification of fruit juices [116].

PE is found in plants, plant pathogenic bacteria and fungi [117]. It has been reported in *Rhodotorula* sp. [118], *Phytophthora infestans* [113], *Erwinia chrysanthemi* B341 [119], *Saccharomyces cerevisiae* [120], *Lachnospira pectinoschiza* [121], *Pseudomonas solanacearum* [122], *Aspergillus niger* [123,124], *Lactobacillus lactis* subsp. *Cremoris* [125], *Penicillium frequentans* [126], *E. chrysanthemi* 3604 [127], *Penicillium occitanis* [128], *A. japonicus* [129] and others.

There are many reports of occurrence of PE in plants, viz., *Carica papaya* [116,130], *Lycopersicum esculentum* [131], *Prunus malus* [132], *Vitis vinifera* [133], *Citrus* sp. [134], *Pouteria sapota* [135], and *Malpighia glabra* L. [136].

3.4.2. Assay methods

PE activity is most readily followed by gel diffusion assay, as described by Downie et al. [137]. Increased binding of ruthenium red to pectin, as the number of methyl esters attached to the pectin decreases, is used as the basis of the assay. The unit of activity in nano- or picokatals is calculated, based on the standard curve generated from the log-transformed commercial enzyme activity versus stained zone diameter. The sensitivity, specificity and simplicity of this PE assay are superior to all others. PE activity can also be measured by using a pH stat because ionization of the carboxyl group of the product releases a proton, which causes a change in pH [109].

3.4.3. Physicochemical and biological properties

PE activity is highest on 65–75% methylated pectin, since the enzyme is thought to act on methoxyl group adjacent to free carboxyl groups [109]. PE action has a very little effect on viscosity of pectin containing solutions unless divalent ions are present, which increase viscosity due to cross-linking. PEs are highly specific enzymes. Some PEs attack only at the reducing chain, while others attack the non-reducing end [23]. The molecular weights of most PEs are in the range of 35–50 kDa (Table 6). pH values at which PEs are active range from 4.0 to 8.0. Fungal PEs have a lower pH optimum than that of bacterial origin. Optimum temperature range for maximal activity for majority of PEs is 40–50 °C. Immobilization studies have also been carried out on PEs [138].

Two isoforms of PE, isolated from *Aspergillus japonicus*, have a pI of 3.8 and are best active at 50 °C [117]. Two different PEs, namely: *PmeA*, an extracellular enzyme [124]; and *PmeB*, an outer membrane protein [139], have been isolated from *Erwinia chrysanthemi*. A PE reported from *Erwinia chrysanthemi* 3937 shows best activity at alkaline pH and 50 °C [127].

3.5. Regulatory aspects of pectinolytic enzymes

The production of pectinolytic enzymes is induced by low concentrations of galacturonic acid. The highest concentration (5%) of the sugar gives lower production levels in stationary phase, indicating that at high concentrations either galacturonic acid or one of its metabolites exhibit self catabolite repression. In the presence of glucose the enzyme production reduces to basal levels [140–142].

4. Biotechnological applications of microbial pectinases

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 work on purification of viruses [143] and in making of paper [144,4]. They are yet to be commercialized.

4.1. Fruit juice extraction

The largest industrial application of pectinases is in fruit juice extraction and clarification. Pectins contribute to fruit juice viscosity and turbidity. A mixture of pectinases and amylases is used to clarify fruit juices. It decreases filtration time up to 50% [27]. Treatment of fruit pulps with pectinases also showed an increase in fruit juice volume from banana, grapes and apples [145]. Pectinases in combination with

Table 6
Biochemical and physicochemical properties of some pectinesterases

Source of PE	Molecular weight (kDa)	pI	K_m	Optimum temperature (°C)	Optimum pH	Temperature stability	pH stability	References
Rhodotorula sp.	–	–	–	40	6.0	50	4.9–9.0	[118]
Erwinia chrysanthemi B341	37	9.6–9.9	–	50	5.0–9.0	–	–	[119]
Aspergillus niger	–	–	1.01	45	5.0	–	–	[124,121]
Apple PE	36	9.0	0.123	60	7.0	75	–	[128]
E. chrysanthemi 3604	37	–	0.03	50	8.0–9.0	–	–	[124]
A. japonicus	46 (PE I)	3.8	–	–	4.0–5.5	50	–	[117]
	47(PE II)	3.8	–	–	4.0–5.5	50	-	

other enzymes, viz., cellulases, arabinases and xylanases, have been used to increase the pressing efficiency of the fruits for juice extraction [146]. Vacuum infusion of pectinases has a commercial application to soften the peel of citrus fruits for removal. This technique may expand in future to replace hand cutting for the production of canned segments [147]. Infusion of free stone peaches with pectinmethylesterase and calcium results in four times firmer fruits. This may be applied to pickle processing where excessive softening may occur during fermentation and storage [147].

4.2. Textile processing and bioscouring 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 [148].

Bioscouring 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 [148].

4.3. Degumming of plant bast fibers

Bast fibers are the soft fibers formed in groups outside the xylem, phloem or pericycle, e.g. Ramie and sunn hemp. The fibers contain gum, which must be removed before its use for textile making [148]. The chemical degumming treatment is polluting, toxic and non-biodegradable. Biotechnological degumming using pectinases in combination with xylanases presents an ecofriendly and economic alternative to the above problem [149].

4.4. Retting of plant fibers

Pectinases have been used in retting of flax to separate the fibers and eliminate pectins [148].

4.5. Waste water treatment

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 [148].

4.6. Coffee and tea fermentation

Pectinase treatment accelerates tea fermentation and also destroys the foam forming property of instant tea powders by destroying pectins [150]. They are also used in coffee fermentation to remove mucilaginous coat from coffee beans.

4.7. Paper and pulp industry

During papermaking, pectinase can depolymerise pectins and subsequently lower the cationic demand of pectin solutions and the filtrate from peroxide bleaching [144,4].

4.8. 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 [148].

4.9. 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 [143].

4.10. Oil extraction

Citrus oils such as lemon oil can be extracted with pectinases. They destroy the emulsifying properties of pectin, which interferes with the collection of oils from citrus peel extracts [151].

4.11. Improvement of chromaticity and stability of red wines

Pectinolytic enzymes added to macerated fruits before the addition of wine yeast in the process of producing red wine resulted in improved visual characteristics (colour 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 [152].

5. Conclusion

Most of the studies performed so far have been concentrated with the screening, isolation, production, purification, characterization and applications of pectinolytic enzymes in increasing the fruit juice yield and its clarification. Some reports are available on the applications of pectinases in other industries. Study of the molecular aspects of pectinases and engineering of enzymes that are more robust with respect to their pH and temperature kinetics by the techniques of protein engineering and site directed mutagenesis should receive increased attention in the coming times. Future studies on pectic enzymes should

be devoted to the understanding of the regulatory mechanism of the enzyme secretion at the molecular level and the mechanism of action of different pectinolytic enzymes on pectic substances.

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