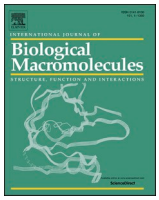
International Journal of Biological Macromolecules 244 (2023) 125385

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac



Sequence, structure and functionality of pectin methylesterases and their use in sustainable carbohydrate bioproducts: A review

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ARTICLE INFO

Keywords:
Pectin methylesterases
Pectin
Plant pathogen
Sequence diversity
Cell wall

ABSTRACT

Pectin methylesterases (PMEs) are enzymes that play a critical role in modifying pectins, a class of complex polysaccharides in plant cell walls. These enzymes catalyze the removal of methyl ester groups from pectins, resulting in a change in the degree of esterification and consequently, the physicochemical properties of the polymers. PMEs are found in various plant tissues and organs, and their activity is tightly regulated in response to developmental and environmental factors. In addition to the biochemical modification of pectins, PMEs have been implicated in various biological processes, including fruit ripening, defense against pathogens, and cell wall remodelling. This review presents updated information on PMEs, including their sources, sequences and structural diversity, biochemical properties and function in plant development. The article also explores the mechanism of PME action and the factors influencing enzyme activity. In addition, the review highlights the potential applications of PMEs in various industrial sectors related to biomass exploitation, food, and textile industries, with a focus on development of bioproducts based on eco-friendly and efficient industrial processes.

1. Introduction

The plant cell wall is a complex matrix composed of multiple networks of carbohydrate polymers, including cellulose, hemicelluloses, pectins, as well as glycoproteins, and, in secondary cell walls, lignin [1]. Pectins are the most complex molecules among these polymers and play a crucial role in the structure and function of plant cell walls [2]. They help maintain the cell wall's mechanical strength and elasticity, regulate water and nutrient movement, act as a barrier against pathogens, and provide structural support and adhesion between cells. The pectin content in plant cell walls varies depending on various factors, such as the plant species, tissue type, developmental stage, and environmental conditions. In general, the primary cell walls of dicotyledonous plants have a relatively high pectin content of approximately 35 %, while grasses typically contain lower levels ranging from 2 to 10 %. Wood tissues are primarily composed of secondary cell walls and contain approximately 5 % pectins, though this can differ depending on the tree species and age considered [3]. Pectin content can also differ within different tissues, with those associated with fruits being known to have the highest content. Three heterogenous pectin polysaccharides have been isolated and characterized from the primary cell walls of plants, including homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II) [4]. Homogalacturonan is the simplest pectin structure, composed of 1,4-linked acidic residues of α-D-galacturonic acid (GalA), where the carboxylic group of some residues is methyl esterified [5]. The degree of methylation (DM) is defined as the percentage of GalA units esterified with methanol. The backbone of rhamnogalacturonan I consists of alternating disaccharide units of 4-linked α-D-galacturonic acid and 2-linked α-D-rhamnose, and is branched with side chains of arabinan, galactan, and arabinogalactan. Rhamnogalacturonan II is the most complex form of pectin, comprising the homogalacturonan backbone with high substitutions of monosaccharide residues, including rhamnose, xylose, arabinose, galactose, and some deoxy sugars such as aceric acid (3-C-carboxy-5-deoxy-l-xylose), KDO (2-keto-3-deoxy-D-manno-octulopyranosylonic acid, and DHA (3-deoxy-D-lyxo-2-heptulopyranosylaric acid), linked together by

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https://doi.org/10.1016/j.ijbiomac.2023.125385
Received 27 April 2023; Received in revised form 8 June 2023; Accepted 12 June 2023
Available online 15 June 2023

20 different glycosidic linkages [6].

Pectins are polymerized, methyl-esterified and substituted in Golgi stacks, and exported into the cell wall in highly methyl-esterified forms [7] (Fig. 1). Highly esterified pectins are modified (i.e., demethylesterification and deacetylation) by the action of pectin degrading enzymes, e.g. pectin methylesterases (PME), producing pectate and releasing methanol [8]. Degradation of the pectin backbone is achieved by the action of three enzyme families: (i) glycoside hydrolases (GH) containing *endo*- and *exo*-polygalacturonases (PG), (ii) esterases (CE) comprising pectin acetyl- and pectin methyl-esterases, and (iii) lyases (PL) containing pectin and pectate-lyase [9]. The hydrolysis by PG requires a water molecule to catalyze the breakdown of a glycosidic bond, whereas PL do not. Moreover, PG and PL have different cleavage specificities; for instance, PG cleaves a linkage between two de-esterified GalA residues, while PL recognizes methyl-esterified GalA [10].

Pectin methylesterases (PMEs) are a group of enzymes ubiquitously found in plants and microorganisms, including bacteria and fungi, and belong to the carbohydrate esterase family 8 (CE8) in the CAZy database (http://www.cazy.org/). During the phytopathogenic process, PMEs are secreted outside the cell along with other carbohydrate-active enzymes [11]. Within the cell wall, PMEs hydrolyze the pectin methyl group at the C6 position of GalA on HG, producing methanol and a negatively charged polymer, along with the release of a proton (H^+). The negatively charged polymer or free carboxylic groups of HG then interact with specific cations, such as Ca^{2+}, to form a so-called egg-box junction that supports the formation of stable pectin gels. The demethylation pattern depends on the PME source and the pH of the system, with PMEs from fungal sources demethylating randomly and PMEs from bacteria/plants demethylating in a block-wise fashion, producing successive demethylated blocks on pectin [12].

Pectins are relevant to various fields, including food technology, nutrition and health, due to their functional properties and potential health benefits [13–15]. The importance of low methoxyl (LM) pectin-based hydrogels with antimicrobial activity and potential use in food packaging has been recognized [14,16], and pectin-based hydrocolloids combined with other biopolymers like cellulose have vast potential in biomedical applications, such as drug or gene delivery and preparation of wound healing dressings. PMEs from plants and bacteria are particularly valuable for industrial and biomedical applications. The demethylation of GalA residues in pectins creates free carboxyl groups that are negatively charged and can facilitate the formation of ionic bonds with positively charged molecules or surfaces. Additionally, PMEs play a pivotal role in cell wall porosity, growth, and development. Many studies discuss the importance of PMEs in plants and industry, while others present their structure-function properties [12,17,18]. This review provides a comprehensive compilation of the structure and sequence diversity of PMEs, and presents their biochemical and kinetic properties. In addition, it provides information about the biological role and potential applications of PMEs.

2. Sources of PME enzymes

PME enzymes from various sources, including plants, insects, fungi and bacteria, have been extensively characterized. The UniProtKB database currently contains 23,116 PME entries with Enzyme Commission number 3.1.1.11, of which only 103 have been characterized as of June 2023. The majority of the reported PME sequences are from plants, followed by significantly lower numbers of microbial enzymes. This contrasts with the Arthropoda and Archaea taxa for which a few PME sequences have been recorded. In addition, some PMEs have been found in plant pathogenic oomycetes, particularly in the genus *Phytophthora* (Fig. 2). Plants typically express several PME isoforms that arise from a multigene family. These isoforms vary in molecular mass, substrate specificity, and action pattern, but all catalyze the same reaction [19].

3. Sequence diversity of PME enzymes

Thousands of PME sequences have been predicted from sequenced genomes; their similarity varies substantially, from 10 to 100 %. Despite this sequence diversity, a pentapeptide motif [LGRPW] (Fig. 3) and some key active site residues are conserved during the evolution of the PME family [5,17,20]. Recently, Kumar et al. performed a comprehensive phylogenetic analysis of PME sequences, which were classified into three main groups based on their similarity: (i) fungal and oomycetes, (ii) bacterial and archaeal, and (iii) plant PMEs [5]. Plant PMEs were found to be more diverse and variable in sequence length than other PMEs [20]. The fungal and oomycete PMEs show significant similarity, albeit with some divergence that allows further separation in two subgroups. Interestingly, some plant PMEs show some similarity with their fungal and oomycete counterparts. Based on sequence information, enzymes from bacteria and archaea are not easily separated. This work also showed that PME from the plant *Vitis riparia* showed more similarity

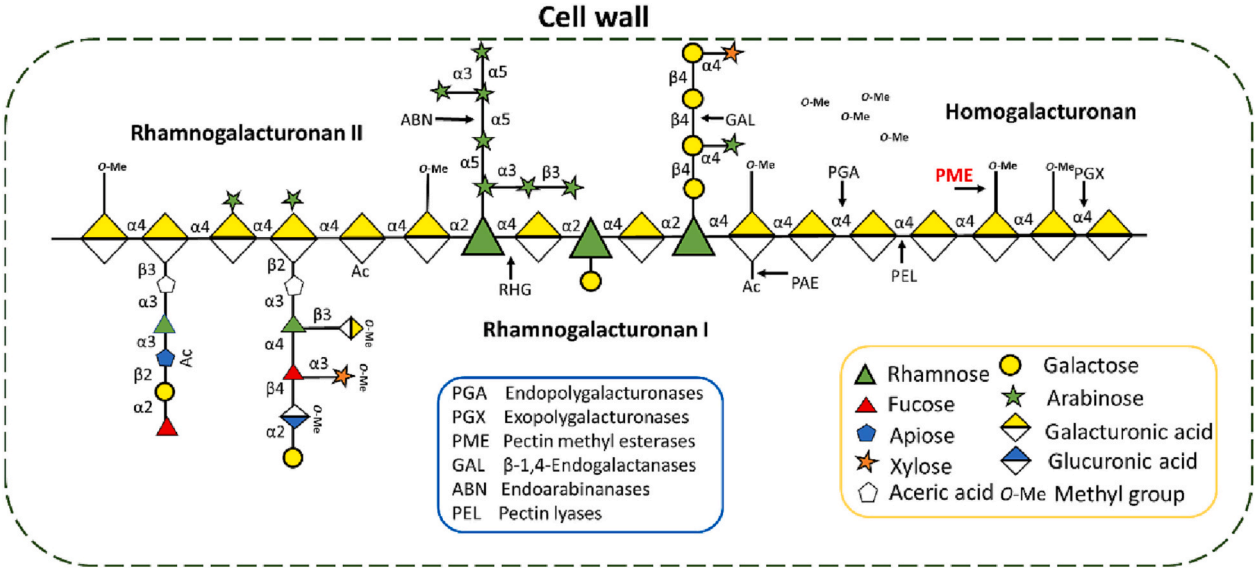

Fig. 1. Schematic structure of pectin showing homogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II regions. The blue box lists pectin degrading enzymes present in the cell wall. Arrows indicate the site of action of the different enzymes.

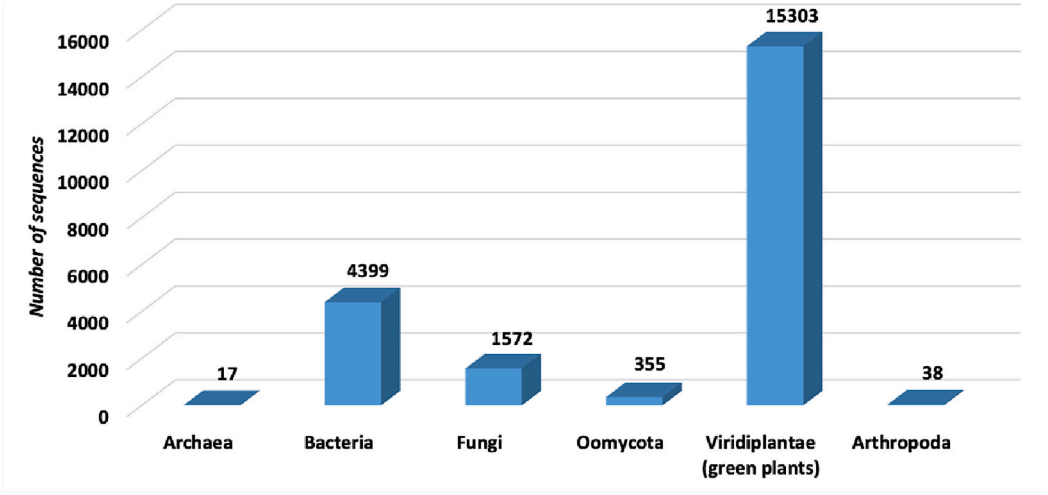

Fig. 2. Total number of PME entries recorded in the UniProtKB database for different taxa.

Fig. 3. Sequence of a PME from Aspergillus niger (UniProt sequence ID: G3YAL0) showing evolutionary sequence conservation with homologous sequences (representative set with 60 % sequence identity). The cyan rectangular box (dotted line) highlights the variable N-terminal signal-peptide region, the green rectangular boxes show conserved motifs, and the red rectangular boxes show active site residues. The figure was generated using the ConSurf Server (https://consurf.tau.ac.il).

with PMEs from the fungus *Phaeosphaeria nodorum* than with other plant PMEs [5].

Experimentally characterized and computationally predicted PMEs have been shown to contain a signature pattern of a pro region/signal peptide at the N-terminal end of the proteins [21–23]. Based on this pattern, genes can be divided into two groups, one containing an extended pro region and 2 or 3 introns, whereas the second group comprises a short or non-existent pro region and 5–6 introns. Evolutionary record analysis of biological sequences reveals that substrate-binding and catalytic residues present at the C-terminal are highly conserved, as are some residues involved in structural maintenance and functionality [5]. Additionally, a tetrapeptide motif [GXXD] is also reported in the PME family (Fig. 3) [5].

4. Structural properties of PMEs

The three-dimensional (3D) structures of PMEs from several organisms have been resolved, with a total of fourteen structures available in the PDB database from seven different organisms (Table 1). The monomeric form of PMEs varies in molecular weight from 25 to 55 kDa and is functionally active. Some eukaryotic PMEs have been found to be glycosylated, with N- or O-linked post-translational glycan modifications [20]. PMEs from bacteria (*Dickeya dadantii* and *Yersinia enterocolitica*), plants (*Solanum lycopersicum* and *Daucus carota*), fungi (*Aspergillus niger*), and insects (*Sitophilus oryzae*) have been structurally characterized [24–29]. The structure of the *D. dadantii* enzyme has been resolved in a co-crystallized form with a hexasaccharide, and another PME structure has been resolved with its inhibitor bound in the active site [30]. Co-crystallization facilitates a better understanding of the enzyme mechanism of action and substrate specificity [30–32]. Sequence identity of these structurally characterized PMEs varies from 25 to 60 %. Although this significant sequence diversity, the core structural topology of these enzymes is superimposable [5]. The resolved structures show a similar fold for all PMEs, which comprises a right-handed parallel β-helix structure (Fig. 4A) [5,17,30]. A comparison of the reported structures of PMEs from different organisms reveals that the loops surrounding the substrate binding site exhibit significant variations, as shown in Fig. 4B.

The 3D structures of the carrot and tomato PMEs are very similar and can be superimposed. The bacterial and fungal PMEs also showed similar structural topologies with their plant counterparts, although they differed in the length of turns extending from the β-helix around the substrate-binding cleft (Fig. 4B). In the bacterial PMEs, these turns were more extended, resulting in a deeper and narrower substrate-binding cleft than in PMEs from other organisms. Notably, loop region of the insect PME near the substrate-binding cleft presents a longer extension with a helix-loop-helix secondary structure. Interestingly, the substrate-binding site of this enzyme was surrounded by aromatic residues,

Table 1
Resolved 3D structures of PMEs from different organisms.

Organism	PDB ID	R-factor (Å-Angstrom)	Remarks	References
Yersinia enterocolitica	3UW0	3.5		[25]
Dickeya dadantii	1QJV, 2NSP, 2NST, 2NT6, 2NT9, 2NTB, 2NTP & 2NTQ	1.7–2.37	D178A mutant and hexasaccharide	[24,30]
Aspergillus niger	5C1C & 5C1E	1.75–1.8	Deglycosylated form	[26]
Daucus carota	1GQ8	1.75		[27]
Solanum lycopersicum	1XG2	1.90	With inhibitor protein	[28]
Sitophilus oryzae	4PMH	1.79		[29]

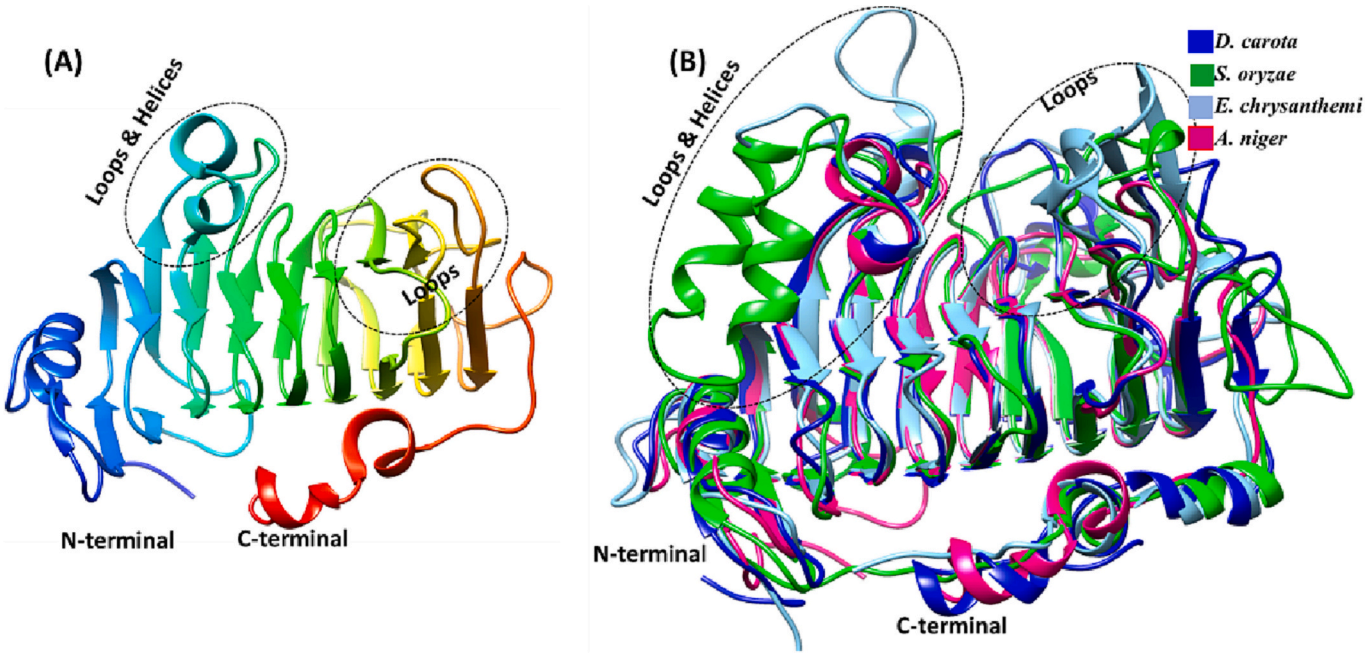

Fig. 4. Structural properties of PMEs. (A) A representative model of the overall 3D structure of a PME. (B) Structural comparison of PMEs from different organisms. Variations in the loop regions are highlighted.

including phenylalanine, tyrosine, and tryptophan. The structure of the bacterial PME was resolved with a methylated hexagalacturonate substrate, providing insight into the catalytic residues involved and reaction mechanism. The positively and negatively charged amino acid composition varies between PMEs, with these amino acids primarily located on the protein's surface. Additionally, the residue arginine is often part of a highly conserved pentapeptide motif [LGRPW] found in the substrate-binding site of PMEs [5]. Comparative analysis has shown that plant and bacterial PMEs tend to have a higher proportion of basic amino acids than fungal PMEs. Within the substrate-binding cleft, positively charged residues such as arginine and lysine play a crucial role in substrate binding through electrostatic interactions [5,30]. However, the specific positive residues involved in this process vary in PMEs from different organisms.

In PME catalytic mechanisms, three conserved acidic residues (aspartate and/or glutamate) are involved: one acts as the nucleophile, another serves as a general acid-base, and the third stabilizes the transition state. Studies on *E. chrysanthemi* PMEs have demonstrated that Asp199 initiates the nucleophilic attack on the C6 ester's carbonyl carbon, while Asp178 functions as a general acid-base catalyst. The acyl intermediate tetrahedral complex is stabilized by Gln174, leading to the release of methanol during catalysis [27].

Recently, a comprehensive molecular modelling study was conducted on a PME from *P. infestans*, which employed systematic molecular dynamics simulations with various methylated pectin substrates, namely fully, partially and de-methylated pentamers of α-(1–4)-linked D-galacturonosyl residues, to explore substrate specificities and binding patterns [5]. The data revealed that the strongest interaction was with partially de-methylated homogalacturonans, indicating a preference for the utilization of this type of substrates. Furthermore, it was demonstrated that both fully methylated or demethylated substrates did not exhibit significant interaction with the substrate-binding site, resulting in their quick release during the simulation [5].

5. Biochemical properties of PMEs

5.1. Characterization of PMEs

PMEs have been identified and studied in various plants and microorganisms. For instance, PMEs have been biochemically characterized in plants such as papaya [33,34,48], apple [35], tomato, orange guava, and lemon [36], potato [37], banana, peaches and strawberries [38]. These plant PMEs have been analyzed for their catalytic efficiency under different reaction conditions. They can be used to modify the pectin composition of fruits and vegetables to produce functional foods [39,40]. Additionally, microbial PMEs have been characterized in species such as *A. niger* [26], *D. dadantii* [24], and *Yersinia enterocolitica* [25]. The characterization of PMEs in both plants and microorganisms has involved the determination of pH and temperature optima and stability, kinetic properties, and the effect of metal ions on enzyme activity. Interestingly, PME isoforms from the same plant can present different biochemical and physical properties, including pH and temperature optima, molecular weight, and isoelectric point. This information is detailed below and summarized in Table 2.

5.2. Effect of pH and temperature on PME activity

PMEs generally have an optimal pH range of 7.0–9.0 [41–45] although some have different pH optima, such as PMEs from papaya (6.5–7.0) [34] and banana (7.0) [46]. A PME from alyanak apricot shows high activity at pH 7.5 [47], while enzymes from *P. infestans,* papaya pulp, and *Datura* have alkaline pH optima of 8.0, 8.5, and 9.0, respectively [5,48,49]. Another PME from *Carica papaya* L. (CpL-PME) has a broad pH optimum range of 6.0 to 9.0 [33] and a PME from *Botrytis cinerea* has an optimal pH of 5.5 and shows maximal activity from pH 2.0 to 8.0 [50]. In terms of pH stability, a PME extracted from *Carica papaya* outer mesocarp-exocarp tissue retains 83 % activity at pH 4–10, and 94 % activity at pH 6.0 after 24 h [34], whereas CpL-PME retains almost 100 % activity for 24 h over a pH range of 3.0–11.0 [33].

Alkaline and acidic PMEs can be used for specific applications. For instance, PMEs are widely used in the food industry to modify texture, consistency and flavor. In this context, alkaline PMEs have applications

Table 2
Summary of different biochemical properties of some PMEs.

S. N.	Organism	Effect of pH		Effect of Temperature (°C)		Effect of metal(s) on enzyme activity		Reference
		Optimum pH	Tolerance/ Stability range	Optimum Temperature	Tolerance/ Stability range	Activation	Inhibition	
1.	*Carica papaya (mesocarp-exocarp tissue)*	7.0	4.0–10.0	60	30–75	NaCl & KCl (essential for EA); $MgCl_2$ and $CaCl_2$ enhanced EA 10 folds	$FeSO_4$, $CuSO_4$, $NiSO_4$ & $ZnSO_4$ inhibited EA	[34]
2.	*Musa acuminata*	7.0	NR	63–64	Up to 65	NR	NR	[46]
3.	*Malus domestica*	6.5–7.5	NR	NR	Up to 40	NR	NR	[35]
4.	*Alyanak apricot*	7.5	7.0–8.0	60	30–40	NaCl (essential for EA)		[47]
5.	*Carica papaya (pulp)*	8.0		60	Up to 60	NaCl (essential for EA)	NR	[48]
6.	*Datura stramonium*	9.0	7.0–10.0	60	Up to 70	NaCl & KCl (for optimal EA)	NR	[49]
7.	*Daucus carota*	7.5	6.5–8.5	55	30–50	NaCl enhanced EA	NR	[41]
8.	*Solanum lycopersicum*	7.0		55	50–80	Ca^{2+} enhanced EA at 300 Mpa pressure	Ca^{2+} decreased EA at Atm. pressure	[52]
9.	*Aspergillus niger ZJ5*	3.8	2.0–6.0	45	20–50	NR	NR	[60]
10.	*Phytophthora infestans*	8.5	NR	45	25–50	NR	NR	[5]
11.	*Erwinia chrysanthemi*	5.0	5.0–9.0	NR	NR	NR	NR	[24]
12.	*Aspergillus niger*	5.0	4.0–4.5	45	NR	NaCl enhanced EA	NR	[26]
13.	*Aspergillus japonicus*	4.5	4.0–5.5	NR	Upto 50	NR	NR	[61]
14.	*Botrytis cinerea*	5.5	2.0–8.0	60	10–80	Na^{+}, K^{+} Mg^{2+} and Ca^{2+} enhanced EA 3–4 folds	NR	[50]

NR = not reported, EA = enzyme activity, Atm = Atmospheric.

in jams, jellies and fruit juices, increasing viscosity and gel formation. Conversely, acidic PMEs can be used in the preparation of pickles, preventing softening and maintaining crispiness.

Studies have examined the temperature optima and thermostability profiles of PMEs from various fruits and fungi. PMEs from *Carica papaya* fruit [34], *Datura* [49], alyanak apricot [47], and the fungus *B. cinerea* [50], showed temperature optima of 60 °C, while PMEs from banana [46] and plum [43] had optima of 63–64 °C and 65 °C, respectively. PMEs from potato [51], black carrot [41] and tomato [52] had an optimum of 55 °C. Some PMEs showed optimal activity at higher temperatures, such as PMEs from Brazilian guava [53] and guava fruit [42], with optima at 70 °C and 90 °C, respectively. A recombinant PME from *P. infestans* had an optimum temperature of 45 °C [5]. In terms of thermostability, purified PMEs from *Carica papaya* [34] and papaya [54] were stable at temperatures up to 60 °C, while a mesostable PME from alyanak apricot [47] was stable in the range of 30–45 °C. A thermostable PME from *Citrus sinensis* fruit retained nearly 100 % of optimal activity at 70 °C after 60 min of treatment [55]. Temperature optima and thermostability profiles of PMEs are crucial for their application in different industries. The optimal temperature range can be exploited for diverse applications, and PME variants can be selected to suit processing conditions and desired product properties. For example, thermostable PMEs with high-temperature optima can be used in high-temperature processes, such as food canning, and pasteurization. PMEs with lower temperature optima and lower thermostability are more suitable for processes that involve refrigeration or freezing, to preserve enzyme activity and maintain product quality.

5.3. Kinetic studies on PMEs

Kinetic studies on PMEs have been conducted to better understand their enzymatic activity, substrate specificity, and mechanism of action. Kotnala et al., determined the Km and Vmax values of a PME from *Carica papaya* using a pectin from citrus as a substrate characterized by 74 % galacturonic acid and 62 % esterification [34]. The Km value was found to be 0.22 mg/mL, while the Vmax value was 1289.15 ± 15.9 units/mg. Previous studies have reported varying Km values for different PMEs. For example, Fayyaz et al. and Vasu et al. reported a Km value of 0.11 mg/mL for a purified PME from papaya and CpL-PME using the abovementioned pectin from citrus [33,56]. The Km values for PMEs from banana and grapefruit were found to be 0.151 mg/mL and 0.274 mg/mL, respectively [46]. In contrast, a low Km value of 0.008 mg/mL was reported for a PME from *Datura* [49], while a high Km value of 0.86 mg/mL was observed for an enzyme from *Arabidopsis* [57]. Similarly, a significantly high Km value of 2.14 mg/mL was reported for a PME from carrot [41]. Unal and Sener reported Km and Vmax values for a PME from alyanak apricot using pectin from apple as a substrate of 1.69 mg/mL and 3.41 units/mL, respectively [47]. A recent study reported that a recombinant PME from *P. infestans* had a Km value of 0.007 % and a Vmax value of 10 μmol/min [5].

5.4. Effect of metal ions on PMEs

Metal ions significantly affect the activity of PMEs, with both positive and negative effects observed depending on the specific metal ion and concentration used. A PME from *Carica papaya* outer mesocarp-exocarp tissue requires monovalent ions for its activity, with optimal enzyme activity obtained in the presence of 0.25 M NaCl or KCl [34]. However, in the absence of these salts, no activity was detected. Interestingly, divalent metal ions (Mg^{2+} and Ca^{2+}) also significantly affected PME activity, albeit at a 10-fold lower concentration than that of the monovalent salts. In another study [33], CpL-PME exhibited optimal activity in the presence of various monovalent salts (NaCl, KCl, and LiCl) at concentrations of 0.15 and 0.3 M. Similarly, PMEs from Valencia orange and alyanak apricot [41] were reported to be activated by NaCl. An earlier study indicated that the activation of PMEs by metal ions does not involve direct interaction [58]. It was reported that the presence of carboxy groups in pectin can impede the enzyme reaction, but the interaction of metal ions with these groups facilitates PMEs' ability to cleave the ester bonds [58,59]. In contrast, some metal ions were found to inhibit PME activity. For instance, the activity of the purified PME from *Carica papaya* was inhibited in the presence of 1 mM of $FeSO_4$, $CuSO_4$ and $NiSO_4$, and 2 mM of $ZnSO_4$, resulting in residual activities of 19 %, 24 %, 41 %, and 62 % (after 5 min incubation), respectively [34]. It has been proposed that the carboxylate groups of pectin adjacent to the ester bond to be cleaved are necessary for PME catalysis [59] and

that masking of carboxylate groups by metal ions may result in the inhibition of PME activity [58,59]. The effect of metal ions on PMEs has important implications for various industrial applications of these enzymes, such as in the food industry, where they are used to modify the texture and rheological properties of foods.

6. Biological function of PMEs

As mentioned earlier, pectins are essential constituents of plant cell walls, affecting various wall properties such as porosity and cell-to-cell adhesion in the middle lamella. The activity of PMEs is regulated by specific inhibitors known as PME Inhibitors (PMEIs), which belong to the Plant Invertase/Pectin Methylesterase Inhibitor Superfamily of proteins [62–65]. The functions of PMEs and their regulation by PMEIs have been extensively studied and reviewed [17,63,66,67].

6.1. PME functions in plants

Plant PMEs are crucial in various aspects of plant biology, including structural and developmental changes, abiotic and biotic stress response, and agronomic traits. During the developmental stages of the plant, PME activity is essential for seed germination and growth, as evidenced by the earlier germination of *A. thaliana* seeds and reduced root hair growth of seedlings in loss-of-function mutants of the PME gene *Atpme3–1* [68]. Additionally, PMEs facilitate mechanical support of the stem by demethylesterifying the primary cell wall, as observed in *A. thaliana* PME35, which directly regulates the strength of the supporting tissue [69]. Moreover, PMEs determine the morphology of the pollen tube [70]. The biological significance of PMEs is not limited to *A. thaliana*, as important functions of genes encoding PMEs have been shown in the development of young vegetative tissues and culm of rice [71], as well as in various tissues of grapevine, including root, stem, tendril, inflorescence, flesh, skins, and leaves [72].

PMEs are also known to play a crucial role in the susceptibility and response of plants to abiotic stress. For instance, the downregulation of PMEs in tomato fruit has been linked to a decrease in Ca^{2+} accumulation in the cell wall and a reduced susceptibility to blossom-end rot (BER), a physiological disorder that can cause severe crop losses [73,74]. Furthermore, studies on PME6 in guard cells have highlighted the involvement of PMEs in stomatal function [75]. In addition, investigations on PME34, PME35, and other related gene products have demonstrated that PMEs have a significant role in heat and drought tolerance by regulating stomatal movement not only in herbaceous plants like *A. thaliana* and soybean but also in woody plants such as poplar [67,76–78].

PMEs play a crucial role in the plant's response to biotic stress. In *A. thaliana*, AtPME17 activity triggers the synthesis of Plant Defensin 1.2 (PDF1.2) through the jasmonic acid-ethylene signaling pathway, which confers resistance to the fungal pathogen *B. cinerea* [79]. In wheat, the expression of several PME genes was associated with resistance to *Fusarium graminearum*, the causal agent of head blight [80]. Apart from their role in biotic stress response, PMEs have also been linked to important traits related to the quality of plant products. For example, genes encoding PMEs are involved in tomato fruit ripening [81] and contribute to the accumulation of ascorbic acid, a beneficial nutrient, in the fruits of tomatoes and other Solanaceae [82–84]. In cotton, PMEs influence the fiber diameter and length [85,86], whereas in navel orange fruits, they are involved in low-temperature-induced sac granulation, which is a physiological disorder that reduces fruit quality by decreasing the soluble solid concentration, sugar, and titratable acidity [85].

6.2. PMEs of plant parasites

Plant parasites utilize various enzymes to degrade the cell wall during infection or for nourishment. One such enzyme is a PME utilized by the insect rice weevil (*Sitophilus oryzae*) to digest the cell wall of cereal grains, along with other enzymes [29]. The plant cell wall is the first line of defense against bacterial and fungal pathogens, which employ cell wall degrading enzymes (CWDEs) to cleave structural polymers of the cell wall and acquire the necessary nutrients for their growth and tissue colonization. Pectin degradation is crucial for successful plant tissue infection, particularly by necrotrophic pathogens [87,88]. Pectic enzymes play a key role in initiating the cell wall degradation process, particularly in plant tissues containing highly methylated pectin (e.g., leaves), where the de-esterification process carried out by PMEs is essential for the action of endopolygalacturonases [88,89] and subsequent pectin depolymerization, which weakens cell wall structures and exposes other polymers to microbial CWDEs. For example, *Phytophthora sojae*, the causal agent of root and stem rot in soybean plants, expresses PME-coding genes, particularly in the first 24 h post-inoculation, indicating their necessity for infection [90]. Consequently, inactivating pectin-degrading enzymes in pathogens can reduce their virulence in host plants [65]. PMEs are also involved in virus-host interactions, especially with viral movement proteins (MPs) [89]. For instance, the tobacco mosaic virus (TMV) requires MP for its dissemination across the entire plant, which has been shown to be associated with a host PME, influencing TMV cell-to-cell movement [91]. The interaction between PMEs and MPs is still not well understood, but one proposed mechanism is that the viral MP binds to the C-terminal domain of an unprocessed PME, thereby facilitating the movement of the viral RNA into the neighboring cell [89]. Additionally, many plant-parasitic nematodes exploit CWDE enzymes during infection to penetrate and migrate through host tissues [89]. Recently, cellulose-binding proteins (CBPs) produced by nematodes were found to interact with plant PMEs, increasing PME activity *in planta* and plant susceptibility to nematodes [92].

7. Industrial applications of PMEs and pectin

7.1. Hydrocolloids

Hydrocolloids are polymers with the ability to form viscous dispersions and/or gels when dispersed in water [93]. As a hydrocolloid, pectin is widely used in the food, beverage, cosmetic, and pharmaceutical industries as a thickening agent and stabilizer, and to encapsulate

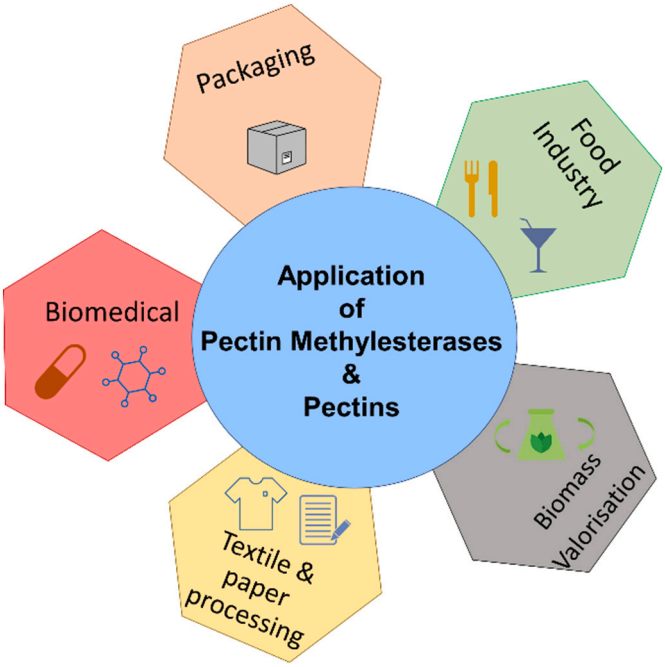

Fig. 5. Major industrial applications of pectins and PMEs.

other compounds (Fig. 5). The degree of methylation in pectin varies depending on the source and extraction process, and its gelification is influenced by temperature, pH, and the presence of cations [94]. There has been a growing demand for low methoxyl (LM) pectin in recent years due to its favorable properties useful in various applications. Indeed, LM-pectin can form water-insoluble gels in the presence of positive divalent or trivalent ions (e.g. Ca^{2+}) over a wide range of pH values and temperatures [95]. However, conventional methods of de-esterification of high methoxyl (HM) pectin through the use of harsh chemicals (e.g. acids, alkali, and alcoholic/aqueous ammonia) [96] are not compatible with high-value products intended for human consumption or use. Therefore, the enzymatic de-esterification carried out by PMEs is considered a sustainable and credible alternative [97]. Recent studies have also explored the possible applications of LM-pectin besides its traditional role as a gelling and firming agent. These include the production of edible films for food packaging [98,99], the preparation of low-calorie/sugar gels in dietetic foods [97], the production of hydrogels for encapsulation and controlled release of drugs, and other biomedical applications such as wound healing and tissue engineering for hard tissue repair [95].

In addition, pectins have many health-promoting effects. Dietary fiber pectins serve as a food source for beneficial gut bacteria which ferment pectin, producing short-chain fatty acids (SCFAs), which support a healthy gut environment and have been linked to improved digestive health and immune function [100]. Moreover, dietary fiber pectins may contribute to maintaining glucose [101] and cholesterol levels [102]. Pectins also bind to heavy metals like lead and cadmium in the digestive tract, reducing the absorption and accumulation of these toxic elements in the body [103].

7.2. Role in the food industry

Pectins are widely used in the food industry (Fig. 5) and contribute to the properties of many food products, e.g., texture, consistency and stability of jams, jellies, fruit fillings, and confectionery items. More specifically, the presence of pectins in fruit juices contributes to their turbidity and consistency, which results in a significant increase in viscosity that hinders industrial processes such as filtration, clarification, and concentration. Exogenous pectic enzymes are commonly used to reduce viscosity and overcome these issues. Typically, commercial preparations contain a mixture of polygalacturonases, pectate lyases, and pectin esterases derived mainly from fungal sources (e.g., *Aspergillus* spp.) [104]. However, the use of PMEs is limited to the clarification of "clear" juices (e.g., apple, pear, and grape), while their presence in commercial products should be avoided in the processing of "cloudy" juices (e.g., citrus, tomato, and nectars), where pure pectin lyases are preferred [104]. The recent developments in understanding PME inhibition opens up new possibilities for mitigating problems arising from undesired endogenous PME activity [65].

Plant and microbial PMEs release methanol through the hydrolysis of methoxy groups in pectin, which is a toxic compound for humans and can lead to acute intoxication and severe health consequences. Unfortunately, nearly all alcoholic beverages obtained from fruit juice fermentation (e.g., wine, apple, hawthorn, and plum) contain methanol [105]. Thus, the activity of PMEs in commercial enzymatic mixtures should be minimized as much as possible [104], and low-pectin varieties should be favored [106].

7.3. Role in biomass exploitation

Plant cell walls possess a complex and heterogeneous structure, which often poses a challenge to the efficient utilization of plant biomass due to their recalcitrance. Consequently, a variety of enzymes are required to synergistically deconstruct the cell wall. The primary cell walls of dicotyledonous species are primarily composed of pectins, which constitute the main interstitial material between cells [107]. The presence of salt bridges formed by methyl groups increases the recalcitrance of cell walls [108]. Thus, the presence of PMEs is crucial for the efficient solubilization and saccharification (i.e. release of polysaccharide-derived sugars by enzymatic hydrolysis) of pectin-rich tissues, where synergism between PMEs and other pectic lyase enzymes has been observed [109]. PMEs have been shown to enhance saccharification and increase the yield of extracted pectin from beetroot residues, thereby providing a potential route for biomass valorization (Fig. 5) [110]. Although industrial processes for this purpose have been proposed [111], no applications are currently available. Furthermore, Lionetti and collaborators demonstrated that the reduction in demethyl-esterified homogalacturonan in *A. thaliana* mutants overexpressing PMEI increased saccharification yield, opening up new perspectives for breeding and developing energetic crops [112].

7.4. Role in textile and paper industries

Preparatory treatments such as degumming and scouring are commonly employed in textile and paper manufacturing processes. However, these procedures often involve high temperatures and alkali treatments, which significantly increase energy consumption and wastewater generation. To address these environmental concerns, researchers have recently investigated the use of alkaline recombinant pectic enzymes, such as PMEs, as a replacement for chemical processes. This approach has shown promising results in effectively replacing traditional methods and presents a new avenue for more sustainable and eco-friendly manufacturing processes (Fig. 5) [113,114].

8. Perspectives

Polymers derived from natural sources are gaining attention as an environmentally friendly and cost-effective alternative to synthetic materials. Despite the well-recognized potential of microbial PMEs in the biotechnology sector, these enzymes remain underutilized for many industrial applications. The number of these enzymes with great industrial potential is expected to increase due to their innate extremophilic properties. Advances in bioinformatics, artificial intelligence, rapid genetic sequencing technology, protein modelling and molecular simulation studies, offer opportunities to design and engineer novel PMEs for industrial purposes. Recent progress in understanding the interaction between PMEs and their inhibitors (PMEIs) has highlighted the importance of this class of enzymes for crop improvement and resistance to environmental stressors. Moreover, large-scale utilization of PMEs and PMEIs could significantly enhance the sustainability of industrial processes and reduce their environmental impact. PMEs are expected to play a crucial role in the production of pectin-based biopolymers for medical applications, thereby positively impacting the biomedical sector. In addition, pectin-based nanoparticles have great potential in oral drug delivery due to their solubility and mucoadhesive characteristics. Recently, high- and low-methoxylated pectin nanoparticles have been studied for their biocompatibility and potential as nanocarrier therapeutics [115]. Overall, continued research on PMEs is likely to lead to new insights into the fundamental biology of these enzymes and their potential applications in various fields.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by a grant from the European Commission (Horizon 2020 FET-OPEN Grant Agreement No. 828940).

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