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Molecular Biology of Microbial Pectate Lyase: A Review

Amit K. Dubey[1], Sangeeta Yadav[1], Manish Kumar[1], Gautam Anand[1] and Dinesh Yadav[1*]

[1]Department of Biotechnology, D. D. U Gorakhpur University, Gorakhpur (U.P.) 273 009, India.

Authors' contributions

This review has been written in collaboration between all authors. Authors AKD, SY, MK and GA have collected informations from the literature on different aspects of pectate lyases, prepared suitable tables and figures and made the first draft. Author DY conceived the idea and finalized the final draft of the manuscript. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Pectate lyase represents an important member of pectinase group of enzymes responsible for the pathogenesis and softening of plant tissues. It also has role in fruit juice clarification and in retting of natural fibers. The biochemical characterization of pectate lyases from diverse microbial sources and plants along with an insight to the protein structure has been dealt earlier but there is a lack of exclusive review on the molecular biology of pectate lyases. This review tries to fill the gap by highlighting the various aspects of molecular biology of microbial pectate lyases especially the cloning and expression of pectate lyase genes from diverse sources attempted so far. The topics covered in this review are a brief description about enzymes associated with degradation of pectin, its classification, applications, updated information about the biochemical characterization of microbial pectate lyases and cloning and expression of microbial pectate lyase genes.

Keywords: Pectin; pectinases; pectate lyases; molecular biology; cloning; genes.

**Corresponding author: E-mail: dinesh_yad@rediffmail.com;*

1. INTRODUCTION

Pectate lyases (E.C 4.2.2.2) also known as pectate transeliminases, depolymerise the pectic acid polymer by β-transelimination mechanism. Pectic acid is de-esterified pectin occurring in the middle lamella of plant cells.Pectic substances are basically heteropolymeric in nature and form the major component of the carbohydrate that is present in the cell wall. These are found as a thin layer of adhesive extracellular material between primary cell wall and adjacent young plant cells.

At the onset of pathogenesis cell wall carbohydrates are first degraded and an array of enzymes are released facilitating the growth and development of the pathogen [1]. Pectinases are the group of enzymes that degrade the pectic substances in different manner depending on the polymer and accordingly specific name is designated on the basis of substrate and mode of action namely polygalacturonases, pectin lyases,pectate lyases,pectin esterases. These enzymes are biotechnologically important industrial enzymes and are produced by a number of microbial sources such as bacteria, yeast, fungi and actinomycetes [2].

Microbial pectinases have been reviewed several times in light of their industrial applications [3-6]. Review of literature indicates that a lot of attention has been paid to microbial polygalacturonases as compared to other members of pectinases. A review solely devoted to microbial pectin lyase was reported few years back [7]. Marin-Rodriguez et al. [8] have reported a short review on plant pectate lyases regarding cell wall degradation ad fruit softening.Areview of microbial pectate lyases describing their role in plant pathogenesis, their phylogenetic relationship and biochemical studies like isozymes,structure,reaction echanism,purification and properties have been reported [9].

The molecular biology tools provide an opportunity to clone required genes coding for industrially important enzyme and further subject it to over expression in suitable host for enhanced production.The availability of genome sequence information of various microbes known to be a potent source of enzymes further reveals the distribution of putative genes coding for respective enzymes which can be fished out using PCR technology.

This review is an attempt to provide comprehensive information about the gene cloning and expression of microbial pectate lyases.

2. ENZYMATIC DEGRADATION OF PECTIN

Pectin is structurally one of the most complex families of polysaccharides constituting 35% of primary walls in dicots and non-graminaceous monocots, 2–10% of grass and up to 5% of walls in woody tissue [10]. It is mostly found in walls of cells surrounding soft parts of the plant, growing and dividing cells, middle lamella and corner of cells [11]. Pectin is ubiquitously observed in cell wall of all higher plants, gymnosperms, pteridophytes, bryophytes and chara, a charophycean alga [12]. It plays a significant role in both primary and secondary wall structure and function [13]. Pectin biosynthesis, plant wall biosynthesis and regulation of cell wall synthesis [14] have been extensively reviewed over the years.

Pectin is reported to have diverse roles in plants such as growth, development, morphogenesis, defence, cell–cell adhesion, wall structure, signalling, cell expansion, wall porosity, binding of ions, growth factor enzymes, pollen tube growth, seed hydration, leaf abscission and fruit development [10,15]. Pectin finds application in food and cosmetic industry where it is often used as a gelling and stabilizing agent.It has multiple positive effects on human health including lowering cholesterol and serum glucose levels, reducing cancer and stimulating the immune response [16,17]. Pectin is also used in the production of a variety of specialty products including edible and biodegradable films, adhesives, paper substitutes, foams and plasticizers, surface modifiers for medical devices, materials for biomedical implantation and for drug delivery [11,18]. Its role in biomass yield and processing for biofuels has recently been reviewed [19].

Pectinases are a complex group of enzymes that degrade various pectic substances (pectin) present in the middle lamella of plant cell wall. These have been classified according to their mode of action and substrates they prefer *viz.* pectinesterases [PE, E.C.3.1.1.11]; polygalacturonases [PG, E.C. 3.2.1.15]; pectate lyases [PL, E.C. 4.2.2.2] and pectin lyases [PNL, E.C. 4.2.2.10]. Mode of action of important pectinases is shown in Fig. 1. They find immense application in clarification of fruit juices, retting of natural fibers, treatment of pectic waste

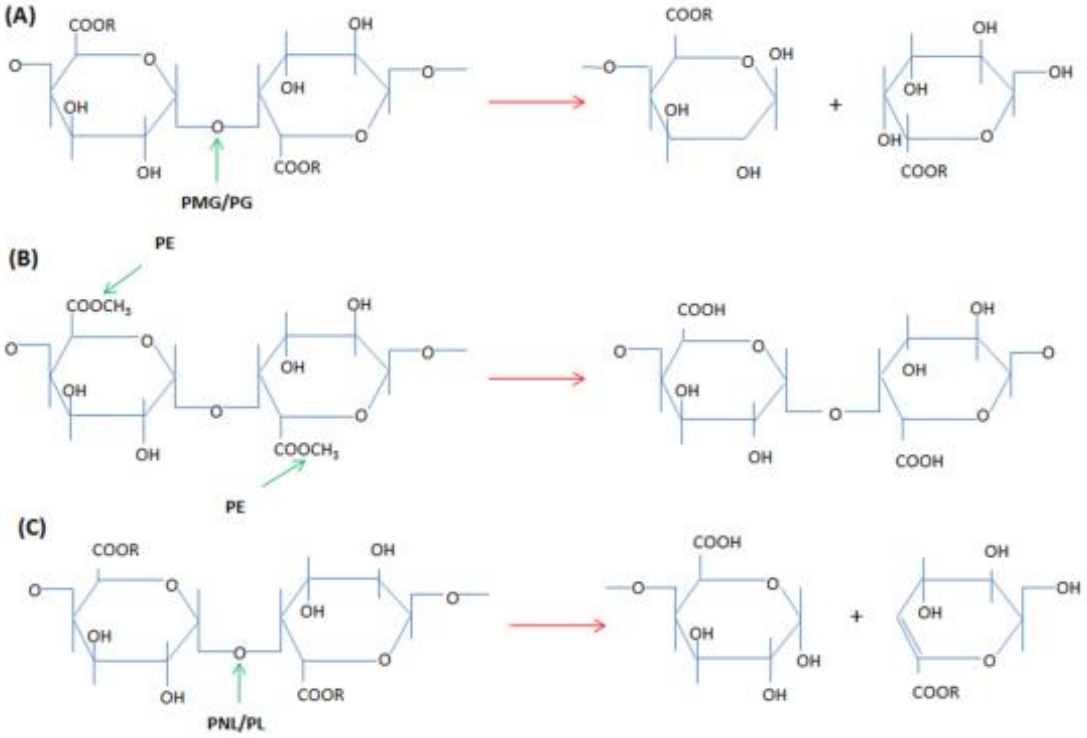

Fig. 1. Schematic representation for mode of action of pectinases (A) R=H for PG (polygalacturonases EC- 3.2.1.15) and CH_3 for PMG (polymethylgalacturonases); (B) PE (pectinesterase EC- 3.1.1.11); (C) R= H for PL (pectate lyase EC-4.2.2.9) and CH_3 for PNL (pectin lyase EC-4.2.2.10). The arrow indicates the place where pectinases react with the pectic substances

water, coffee and tea leaf fermentation, oil extraction, virus purifications, developing functional foods etc. [20-23,4-6].

3. CLASSIFICATION OF PECTINASES

The great complexity and diversity in the smooth and hairy regions of pectin require several kinds of degrading enzymes based on the specificity of substrate as well as type of reactions they catalyse. The group of enzymes which are involved in the degradation of hairy region of pectins are rhamnogalacturonan hydrolase (RG hydrolase), rhamnogalacturonan lyase, rhamnogalacturonan rhamnohydrolase (RG rhamnohydrolase), rhamnogalacturonan galactohydrolase (RG galactouronohydrolase). There are only few reports about this group of enzymes [24,25].

There are, however, other enzymes involved in degradation of side chains of pectins which include α-arabinofuranosidase [E.C 3.2.1.55], endoarabinase [E.C 3.2.1.99], β-galactosidase [E.C 3.2.1.23], endogalactanase [E.C 3.2.1.89] and feruloyl and p-coumaroyl esterases [26]. There is a need for extensive studies on these groups of enzymes targeting their structural and functional aspects so as to explore its industrial applications. Some of these enzymes are discussed below briefly.

3.1 Pectinases Degarading Hairy Region

3.1.1 Rhamnogalacturonan Hydrolases (RG)

These enzymes randomly hydrolyse the rhamnogalacturonan chain producing oligogalacturonates [27]. RG-hydrolase hydrolyses the α-1, 2-rhamnose linkage of galacturonic acid to release oligosaccharides with rhamnose at the non-reducing end.

3.1.2 Rhamnogalacturonan lyases (RG lyases)

(EC 4.2.2.-): These cleave the 'rhamnose α-1,4-galacturonic acid' linkage to release an

unsaturated galacturonate at non-reducing end of the oligomer and a second oligomer containing a rhamnose as a reducing end residue [25]. These enzymes are classified into polysaccharides-lyase families 4 and 11.

3.1.3 Rhamnogalacturonan (RG) rhamnohydrolase (EC 3.2.1.40)

These are also known as rhamnogalacturonan α-L-rhamnopyranohydrolase or α-L-rhamnosidase.Theycarry out the hydrolysis of rhamnogalacturonan at the non-reducing end producing rhamnose [28]. These enzymes are classified into glycosyl-hydrolase families 28, 78, 106.

3.1.4 Rhamnogalacturonan glacturono-hydrolases (EC 3.2.1.-)

Produces monogalacturonate by the hydrolytic cleavage of rhamnogalacturonan chain at the non- reducing end. It is classified into glycosyl-hydrolase family 28.

3.1.5 Rhamnogalacturonan acetylesterases (C 3.1.1.-)

Carries out hydrolytic cleavage of acetyl groups from rhmnogalacturonan chain. It is classified into carbohydrate esterase family 12.

3.1.6 Xylogalacturonan hydrolasee (EC 3.2.1.-)

Produces xylose-galacturonate dimers by hydrolytic cleavage of glycosidic linkages between two galacturonate residues in xylose-substituted rhamnogalacturonan chain [29]. These enzymes are also classified into glycosyl-hydrolase family 28.

3.2 Pectinases Degrading Smooth Region of Pectin

The group of enzymes which are associated with the degradation of “smooth region” (homogalacturonan) can be broadly categorized in two groups namely esterases and depolymerases. The detail classification of pectinases is shown in Table-1.

3.2.1 Esterases (PME/PAE)

This group of enzymes are basically deesterifying enzymes which remove methoxyl and acetyl residues of pectin to produce polygalacturonic acid. It includes pectin methyl esterases [PME, E.C 3.1.1.11] and pectin acetyl esterase [PAE, E.C 3.1.1.6].

3.2.1.1 Pectin Methyl Esterase (PME)

Pectin methyl esterase or pectin esterase (EC 3.1.1.11) catalyzes de-methylesterification of pectin forming pectic acid and methanol. The enzyme acts preferentially on a methyl ester group of galacturonate unit next to a non-esterified galacturonate unit releasing methanol and proton, creating negatively charged carboxyl groups. It acts prior to polygalacturonase and pectate lyases, which need non-esterified substrates [30].

It is classified into carbohydrate esterase family 8, CE8 [31]. PME and its proteinaceous inhibitor [32] along with an insight to structure and function [33] have been reviewed.

3.2.1.2 Pectin Acetyl Esterase (PAE)

Pectin acetyl esterase (EC 3.1.1.-) hydrolyses the acetyl ester in the homogalacturonan region of pectin forming pectic acid and acetate [34]. It is classified into carbohydrate esterase families 12 and 13 [31].

3.2.2 Depolymerases

The other subclass of smooth region (homogalacturonan) degrading group are broadly termed as depolymerases which break the α-1, 4 linkages either by hydrolysis i.e. polygalacturonases [PG, E.C 3.2.1.15] or *via* transelimination mechanism namely pectate lyases [PL, E.C 4.2.2.2] and pectin lyases [PNL, E.C 4.2.2.10].

3.2.2.1 Polygalacturonases (PG)

Polygalacturonases catalyzes hydrolysis of α-1,4-glycosidic linkages in polygalacturonic acid (PGA) producing D-galacturonate. It is essentially a hydrolase classified into glycosyl-hydrolases family 28 [35]. Based on the mode of action it is further classified as Endo-PG and Exo-PG.

3.2.2.1.1 Endo-PG (EC 3.2.1.15)

Endo-PG hydrolyses PGA in a random fashion and liberates saturated oligogalacturonides and galacturonic acid. Endo-PGs are produced by numerous fungi and yeast, higher plants and some phytoparasitic nematodes [36].

Table 1. Classification of pectinases

Enzymes	E. C. Number	Mode of action	Action pattern	Primary substrate	Product
Esterase					
Pectin methyl esterase	3.1.1.11	Hydrolysis	Random	Pectin	Pectic acid+methanol
Depolymerase					
A. Hydrolases					
Protopectinases		Hydrolysis	Random	Protopectin	Pectin
Endopolygalacturonase	3.2.1.15	Hydrolysis	Random	Pectic acid	Oligogalcturonates
Exopolygalacturonase	3.2.1.67	Hydrolysis	Terminal	Pectic acid	Monogalacturonate
Exopolygalacturonan-digalacturonohydrolase	3.2.1.82	Hydrolysis	Penultimate bonds	Pectic acid	Digalacturonates
Oligogalacturonate hydrolase		Hydrolysis	Terminal	Trigalacturonate	Monogalacturonates
Δ4:5 Unsaturated oligogalcturonate hydrolases		Hydrolysis	Terminal	Δ4:5(Galacturonate)$_n$	Unsaturated Monogalacturonates & saturated (n-1)
Endopolymethyl-galacturonase		Hydrolysis	Random	Esterified pectin	Oligomethylgalacturonates
Exopolymethyl-galacturonase		Hydrolysis	Terminal	Esterified pectin	monogalacturonate
B. Lyases					
Endopolygalacturonate lyase	4.2.2.2	Trans-elimination	Random	Pectic acid	Unsaturated Oligogalacturonates
Exopolygalacturonate lyase	4.2.2.9	Trans-elimination	Penultimate bonds	Pectic acid	Unsaturated digalacturonates
Oligo-D-galactosiduronate lyase	4.2.2.6	Trans-elimination	Terminal	Unsaturated digalacturonates	Unsaturated monogalacturonates
Endopolymethyl-D-galactosiduronate lyase	4.2.2.10	Trans-elimination	Random	Unsaturated Poly-(methyl-D-digalacturonates)	Unsaturated methyloligogalacturonates
Endopolymethyl-D-galactosiduronate lyase		Trans-elimination	Terminal	Unsaturated Poly-(methyl-D-digalacturonates)	Unsaturated methylmonogalacturonates

3.2.2.1.2 Exo-PG (EC 3.2.1.67)

It catalyses the hydrolytic release of saturated galacturonic acid residue from non-reducing end of homogalacturonan. Exo-PGs are produced by bacteria and fungi. Two types of exo-PG are identified namely fungal and bacterial exo-PGs. Fungal exo-PGs produce monogalacturonic acid as the main end product and have pH optima of 4.0-6.0 [36]. This enzyme is also called galacturan 1,4-α-galacturonidase or exo-PG 1. However, bacterial exo-PG enzymes produce digalacturonic acid as the main end product. They are mostly designated as exo-polygalacturonidase or exo-PG 2.

3.2.2.2 Pectin Lyase (PNL)

Pectin lyase (EC 4.2.2.10) cleaves pectin by β-elimination mechanism that results in the formation of 4, 5 unsaturated oligogalacturonates without affecting the ester content of the polymer chain, which is responsible for specific aroma of fruits. It does not produce methanol which is toxic and hence preferred in fruit juice clarification industries. For the first time, the application of alkaline pectin lyase in retting of natural fiber was elucidated [37]. An exclusive review highlighting the diverse sources, purification and characterization of pectin lyases from different sources and molecular biology of pectin lyases has recently been published [7].

4. APPLICATION OF PECTINASES

Pectinase production shares about 10% of the overall manufacturing of enzyme preparations [3]. On the basis of their biochemical properties, pectinase have been utilized in number of industries. Acid pectinases are used in clarification of fruit juices, maceration of vegetables in the production of pastes and puress, wine making etc. Alkaline pectinase finds application in processing of natural textile fibers (such as jute, flax and hemp), treatment of pectic waste water, coffee and tea fermentation, vegetable oil extraction, treatment of paper pulp etc. The diverse applications of pectinases are summarized in Fig. 2.

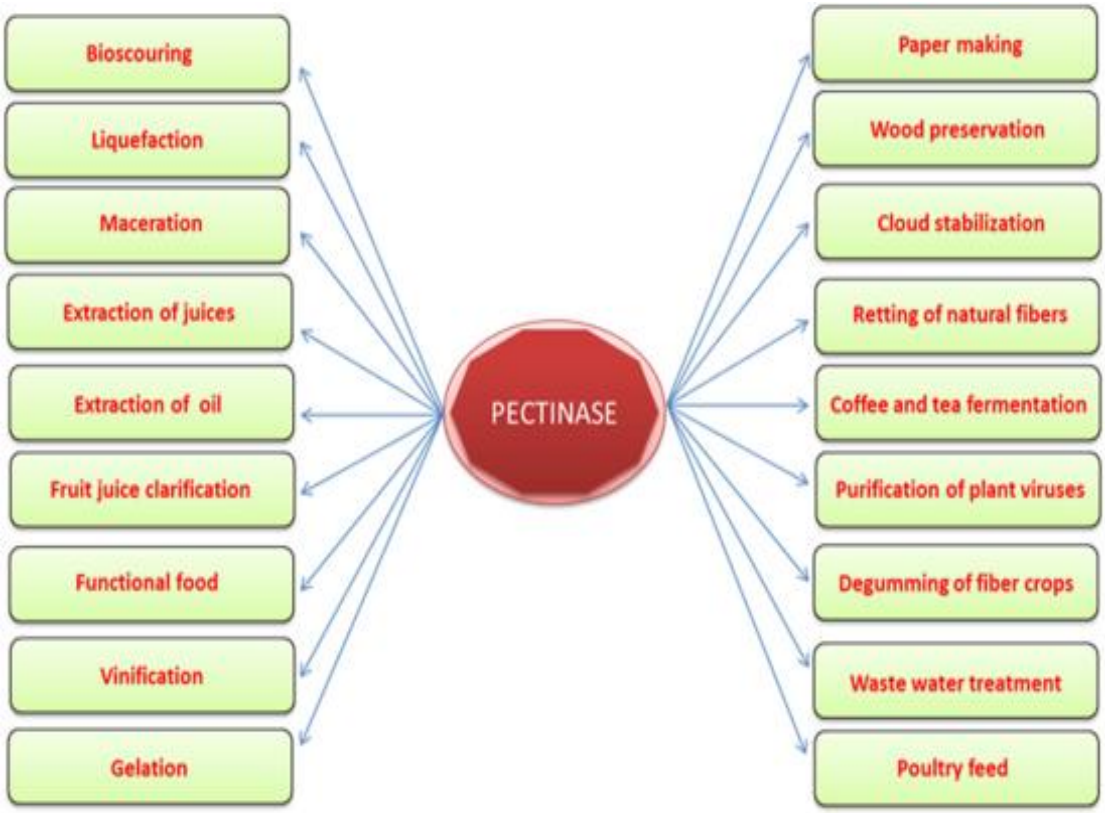

Fig. 2. Pictorial representation of diverse application of pectinases

5. PECTATE LYASES

Pectate lyase (PL) acts on de-esterified pectin (pectate) and cleaves galactosidase linkage forming unsaturated product 4,5-D galacturonate by trans-elimination reaction. On the basis of random or sequential cleavage of α -1,4 glycosidic links in pectic acid (polygalacturonic acid), pectate lyase can be categorized as Endo PL (4.2.2.1) and exo PL (4.2.2.2). Cleavage by PL requires calcium ions, hence it is strongly inhibited by chelating agents such as EDTA [21]. PLs are mostly secreted by plant pathogens but the abundance of PL like sequence in some plant genomes such as *Arabidopsis* strongly suggests an important role in various plant developmental processes [8,4]. The microbial pectate lyases are preferred for the bioscouring applications and directed evolution of a novel pectate lyase for processing cotton fabric, ramie degumming has been attempted [38,39]. Biochemical characterization of microbial pectate lyases along with protein structure has recently been reviewed [4]. Pectate lyase is widely distributed in diverse families of microorganism and several pectate lyases have been reported from bacteria, actinomycetes, fungi and yeasts.

5.1 Purification and Characterization of Pectate Lyases from Different Microbial Sources

The purification and biochemical characterization of microbial pectate lyases from diverse sources have been reviewed earlier. An updated list of the microbial pectate lyases produced from different sources with detail about the purification strategies and biochemical characteristics reported so far is shown in Table 2.

5.2 Cloning and Expression of Microbial Pectate Lyases

There exists great diversity of pectate lyases genes and several pectate lyase genes have been cloned from diverse sources both microbial and plants. The *in-silico* characterization of pectate lyase protein sequences from different microbial sources for homology search, multiple sequence alignment, phylogenetic tree construction and motif analysis has recently been reported [40]. The extensive list of cloned pectate lyase genes mainly from bacterial and fungi is provided in Table 3 and detail is discussed. The diversity of cloned pectate lyase genes mainly from bacterial and fungal sources is shown by constructing phylogenetic tree based on the translated protein sequences (Fig. 3).

5.2.1 Bacterial pectate lyase

Pectate lyase genes from bacteria *Erwinia chrysanthemi* and *Erwinia caratovora* have been extensively characterized. The introduction of the cloned pectate lyase genes from *Erwinia chrysanthemi* namely *pel*B or *pel*E into *Escherichia coli* resulted in the development of blackleg disease symptoms in potato similar to what has been observed by the infection of the pathogen *Erwinia carotovora* [41]. Heterologous expression of an *Erwinia carotovora subsp, atroseptica* pectate lyase 3 (*PL*3) genes in *Aspergillus niger, A. nidulans and A. awamori* have been reported [41]. A total of seven putative pectate lyase clones were screened from the genomic library of *Erwinia chrysanthe*mi EC16 [43]. These seven clones secreted PL into the periplasm or extracellular fluid. It was suggested that gene for the enzyme of pI 9.8 might be nontandemly repeated on the chromosome because its clone was obtained more frequently from the library. Expression studies of subclones of plasmids in *E. coli* suggested that translation signals and signal peptide sequences of *E. chrysanthemi* genes functions well in *E. coli*. Gene annotation information from *Dickeya chrysanthemi* isolated from a recreational lake reveals the presence of multiple copies of pectate lyase in different contigs [44]

The *pel*B and *pel*E genes of *Erwinia chrysanthemi* EC16 have been over expressed in *Escherichia coli* host cells [45]. These genes coded for enzyme with similar physical properties *viz.*molecular weight 40 kDa. These enzymes were predominantly secreted in the periplasm than in the culture medium. A purine-rich Shine-Delgarno sequence with an internal AGGA is located at base 241 and is appropriately positioned 5' to the presumed translational start codonat base 253 of *pel*E. *Erwinia chrysanthemi* pelE sequence beginning at base 103 showshomology in 9 of 13 positions with the consensus *E. coli* catabolite activator protein binding sequence and contains a properly positioned obligatory G(T/A)G sequence at base 107. An eleven base palindromic sequence occurs at positions 168 through 178, downstream from the putative promoter and catabolite activator protein-binding sites. This sequence could be an operator sequence regulating peLE production by a trans-acting element produced by *E. chrysanthemi* EC16.

The sequence study of *pel*B has shown that the presumed ATG translational start codon at base 714 is favorably positioned behind a Shine-Delgarno box with the same internal AGGA sequence as the pelE gene.A sequence beginning at base 516 is expected to function as a strong promoter in *E. coli*, and a possible catabolite activator protein-binding site is present beginning at base 558. The 11-base palindromic sequence observed in the pelE gene is not present in the 5' region of the pelB gene.

Few pectate lyase genes from *Erwinia chrysanthemi* EC16 have been cloned in vectors such as pUC9 plasmid and phage lambda. These genes are closely located on the chromosome and are expressed constitutively but accumulate in periplsasm rather than secreted into the culture medium [46]. The *pel*C gene of *E. chrysanthemi* EC16 with single ORF shows considerable homology to *pel*B gene.Significant homology is not found between the 5' noncoding DNA of *pel*C and that of *pel*B, but possible promoter elements are present in both genes. No identifiable catabolite repressor-binding site was present in the 5' DNA of *pel*C.The predicted molecular weight of the PLc preprotein is 39.9 kDa and that of the mature protein is 37.6 kDa.The *pel*A gene codes for mature enzyme of 361amino acid with calculated molecular weight of 38.7 kDa. The corresponding gene has ShineDalgarno sequence just before assumed start codon at position 1099. 5' untranslated end of the gene has unusually long stretches of AT-rich DNA. The gene is terminated with TAA stop codon and a GC-rich palindromic sequence followed by a T repeat is found after the translational stop at positions 2295 to 2320[47].

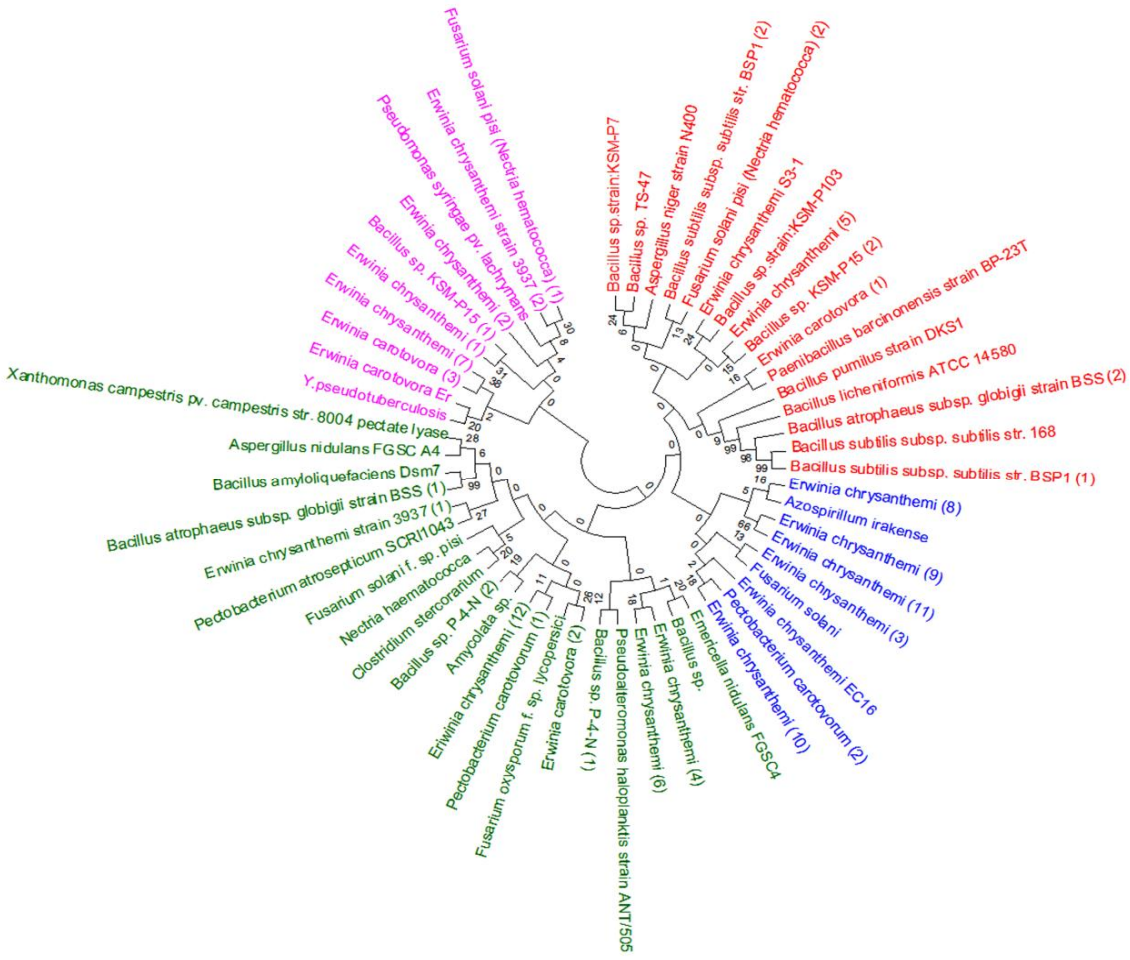

Fig. 3. Phylogenetic tree showing diversity of cloned pectate lyase genes

Table 2. Biochemical properties of microbial pectate lyases

Sl. no.	Source	Purification strategy	Optimum pH	Optimum temp.	Km	Molecular weight	References
1	*Erwinia carotovora*	Ion-exchange chromatography	8.3	40		44	[104]
2	*Erwinia chrysanthemi* EC16		7.5-8.0			76	[105]
3	*Bacillus subtilis* SO113		8.4	40	0.86	42	[106]
4	*Bacillus macerans*		9.0	60	------	35	[107]
5	*Erwinia chrysanthem*i Pel A		8.6	50	0.43	44	[108]
6	*Amycolata sp.*		10	70	0.02	31	[109]
7	*Fusarium solani f.* sp.*pisi*	Gel filtration	10.0			29	[90]
8	*Fusarium solani f.* sp.*pisi*	Mono Q anion exchange	9.5	55		26	[89]
9	*Fusarium moniliformae*		8.5	50	1.2	12.1	[110,111]
10	*Pseudomonas fluorescens*		8.5-9.5	46-52	1.28	41-42	[112]
11	*Thermomonospora fusca*		10.5	60	0.5	56	[113]
12	*Erwinia chrysanthemi*						
	Pel A		8.5	55	0.03	42.5	
	Pel B		9.3	60	0.02	39.5	[50]
	Pel C		9.2	60	0.28	39.5	
	Pel D		8.8	50	0.42	42.0	
	Pel E		8.0	50		42.5	
13	*Bacillus sp.* KSM-P103		10.5	60-65		33.3	[58]
14	*Bacillus sp*.Ksm P7	DEAE coloumn chromatography	10.5	65		33	[114]
15	*Bacillus sp*.Ksm P15	DEAE coloumn chromatography	11.5	55		70	[61]
16	*Bacillus sp* KSM-P-15		10.5	50-55		33	[60]
17	*Bacillus sp*.P-4-N	DEAE coloumn chromatography	11	50		34	[59]
18	*Thermo anaerobacter italicus* sp.	Ion-exchange & Hydrophobic chromatography	9.0	80	0.5	135	[115]
19	*Bacillus* sp.TS 47		8.0	70	----	50	[116]
20	*Bacillus* sp.BP-23		10.0	50		23.2	[55]

Sl. no.	Source	Purification strategy	Optimum pH	Optimum temp.	Km	Molecular weight	References
21	*Clostridium cellulovorans cellulosome*		8.0	75	-----	42	[117]
22	*Pseudoalteromonas haloplanktis*	Anion exchange	9-10.0	30 30	1 g/L 5 g/L	68 75	[77]
23	*Pseudomonascellulose*		10	62	0.104	68.5	[76]
24	*Bacillus* sp.P4-N		11.5	70	----	35	[118]
25	*Bacillus alcalophillus*		9-10	45	0.08	35	[119]
26	*Thermotoga maritima*	Ion exchange chromatography	9.0	90	0.06	40	[75]
27	*Bacillus licheniformis*		8.5	70	0.56	33.4	[57]
28	*Mrakia frigid*		8.5-9.0	30			[120]
29	*Bacillus pumilus*BK2		8.5	70	0.24	37.3	[121]
30	*Bacillus subtilis* 168		10.0	65	0.15	23	[56]
31	*Aspergillus nidulans*	Ni-Nitriloacetate agarose coloumn	10	50	0.50	55	[99]
32	*Bacillus sp*.N16-5	DEAE Sepharose coloumn & Ion exchange	11.5	50		42	[68]
33	*Bacillus sp*.	Ion Exchange chromatography	9.0	60	0.025	42	[67]
34	*Streptomyces thermocarboxydus*	Ni-Nitriloacetate agarose coloumn	------	---------	------	23	[122]
35	*Phytophthora capsici*	Affinity & gel filtration chromatography				44	[123]
36	*Pectobacterium cartovorum*	----------	10	70	0.4	-----	[124]
37	*Bacillus subtilis*	Ni-Nitriloacetate agarose coloumn	9.5	50	0.09	46	[66]
38	*Bacillus subtilis* 7-3-3	----------	9.5	50	----------	25	[125]
39	*Bacillus stearothermophilus* *Bacillus cereus* *Bacillus subtilis*		7.5 8.0 9.0	60 50 50			[126]
40	*Dickeya dadanti*	----------	7.4	50	2.5	43	[127]
41	*Volvariella volvacea*		10.0	60	0.681		[128]
42	*Geogenia muralis*	Anion exchange & gel filtration chromatography	10.0	50		51	[129]

Table 3. List of cloned microbial pectate lyase genes

S. no.	Source	Gene	Vector	Host	Molecular mass of recombinant PL (kDa)	Characteristics of recombinant PL	References
1	*Erwinia chrysanthemi*	*PL*	pBR329,pHC79	*E. coli*	39	pI=9.8, pH=8.5	[43]
2	*Erwinia chrysanthemi*	*PL*	pBR322	*E. coli* HB101	Multiple forms	pI=7.8	[130]
3	*Erwinia caratovora EC 14*		pBR322	*E. coli*		pI=9.5	[131]
4	*Erwinia caratovora sub sp atroseptica*		pBR322		31	pI=9.2	[132]
5	*Erwinia caratovora*	*Pel B*	pSH2111, pUC8	*E. coli* HB101	44	pH=8.3, OT=40	[104]
6	*Erwinia chrysanthemi (EC 16)*	*Pel A,Pel B, Pel C, Pel E*	pAKC311, pAKC312, pAKC313,pAKC34				[49]
7	*Erwinia chrysanthemi EC16*	*Pel A, Pel C*	pINK1	*E. coli*	Pel A-45 Pel C-39		[47]
8	*Yersinia pseudotuberculosis*	*PL Y*	pUC, pPELY	*E. coli* DH5α	55	pI=4.5	[71]
9	*Erwinia caratovora*	*PAL I, PAL II*	pNN1, pNN101	*E. coli* HB101	37.5		[133]
10	*Erwinia chrysanthemi EC16*		pUC19,pBR322, pUM24	*E. coli.* DH5α		pH=7.5-8	[105]
11	*Erwinia caratovora Er*	*PelIII*	pUC18,pBR322	*E. caratovora, E. coli* HB101		pH=7.5	[134]
12	*Pseudomonas fluorescens*	*Pel*	pBR 322,pROTM2	*E. coli* HB101			[73]
13	*Bacillus subtilis*		pT7-5,pT7-6 pNP111,pNP112	*E. coli*	42		[135]
14	*Pseudomonas marginalis*	*Pel*	pUC119	*E. coli* DH5α	40.8		[74]
15	*Erwinia chrysanthemi 3937*	*PelL*					[51]

S. no.	Source	Gene	Vector	Host	Molecular mass of recombinant PL (kDa)	Characteristics of recombinant PL	References
16	*Erwinia chrysanthemi 3937*	*PelZ*					[52]
17	*Erwinia caratovora atroseptica*	*EcoPL3*	pGW1100 pGEM-72f(+)	*Aspergillus niger, A. awamori, A. nidulans*	60		[42]
18	*Erwinia chrysanthemi 3937*	*Pel I*	pULB110 p365T,pT406, pT7-6		34	pI=9, pH=8.5,OT=37°C	[34]
19	*Pseudomonas syringae PV lachrymans*	*Pel s*	pCPP34,pCPP47	*Pseudomonas syringae* PV BUVS1		pI=9.4	[75]
20	*Amycolata sp.*	*Pel*	pIJ702	*Streptomyces lividans* TK 24	30		[78]
21	*Erwinia chrysanthemi 3937*	*Pel A to Pel E*	pT7-5, pT7-6	*E. coli NM522*		pH O.T.(°C)	[50]
				E. coli BL21(DE 3)	Pel A 42.5	Pel A 8.5 55	
					Pel B 39.5	Pel B 9.3 60	
					Pel C 39.5	Pel C 9.2 60	
					Pel D 42	Pel D 8.8 50	
					Pel E 42.5	Pel E 8 50	
22	*Bacillus sp.KSM-P103*	*Pel103*	pUC18, pHSP64	*E. coli* HB101	33	pI=10.5,pH=10.5, 60-65°C	[58]
23	*Azospirillum irakense*	*Pel A*	pUC18 pLAFR3	*E. coli*		pH=9,OT=37°C, Km=0.076 mg ml^{-1} Vmax=23 µmol ml^{-1}	[79]
24	*Erwinia chrysanthemi 3937*	*PeLX*	pUC18, pBSAp, pBSCm, pT7-5	*E. coli* NM522 *E. coli* BL21(DE3)			[34]
25	*Bacillus sp. KSM-P15*	*Pel-15H*	pHSG398	*E. coli* HB101	69.5		[61]
26	*Bacillus sp P-4-N*	*Pel-4A*	pUC18,pHY300PLK	*Bacillus subtilis* ISW1214	34	pI=9.7, pH=10.5, OT=30°C	[59]

S. no.	Source	Gene	Vector	Host	Molecular mass of recombinant PL (kDa)	Characteristics of recombinant PL	References
27	*Bacillus sp. BP23*	*PelA*	pBR322	*E. coli* 5K	23.2	pH=10,OT=50°C,satbilty pH=4-8,stable temperature=40-60°C	[55]
28	*Bacillus sp. KSM P-15*	*Pel 15E*	pUC18	*E. coli* HB101, *Bacillus subtilis* ISW1214	33	pH=10.5	[60]
29	*Bacillus sp* TS47	*BsPel*	pBluescript II SK+ ,pUBPL 47	*B.subtilis*	50	70°C	[136]
30	*Pseudomonas cellulosa*	*Pel 10A*	λZAPII	*E. coli* XL1Blue *E. coli* BL21 *E. coli* XLOLR	38	pH=10 OT=62°C	[76]
31	*Pseudoalteromonas haloplanktis ANT1505*	*PelA* *PelB*	pRSET-A	*E. coli* DH5α *E. coli* BL21	68, 75	pH=9-10, OT=30°C Km=1 mg ml^{-1}	[77]
32	*Bacillus subtilis IFO3134*		pET22b	*E. coli*	45.4	pH=7, pI=8.3,OT=50°C	[62]
33	*Treponema pectinovorum*	*Pel A*	pLAFR5(λ phage), pBluescript	*E. coli*. DH5α	48.172	pH=8.6, OT=37°C	[80]
34	*Thermotoga maritima MSB8 (DSM 3109)*	*PelA*	pET24d	*E. coli* TG1, *E. coli* BL21		Km=0.06,pH=9,OT=90°C	[81]
35	*Bacillus licheniformis* 14A	*PelA*		*E.coli*	33.4	Km=0.56 g/l, Vmax=51µmol/min	[57]
36	*Bacillus subtilis*	*Yup A*	pJF118HE,pET28a	*E. coli* 5K, *E. coli.*BL21	24.2	pH=10, OT=65°C,pI=8.85	[56]
37	*Bacillus subtilis WSH B04-02*		pET22b, pHsh	*E. coli* JM109	43	pH=9.4,OT=50°C	[63]
38	*Bacillus subtilis*		pPIC9K	*Pichia pastoris* GS115	43.6	pH=9.4 OT=65°C	[65]
39	*Xanthomonas compestries*	*PLxc*	pSD80	*E. coli* Rosetta 2		pH=8.5, OT=50°C	[83]
40	*Bacillus subtilis*	*PEL 168*	pET28a,	*Pichia pastoris*	48.6,	pH=9.5, opti	[66]

S. no.	Source	Gene	Vector	Host	Molecular mass of recombinant PL (kDa)	Characteristics of recombinant PL	References
			pHBM905A		51.4	temp=50°C,pH=9.5, Km=0.09 mg ml^{-1}, Vmax=18.13 µmol ml^{-1}	
41	*Xanthomonas compestris PV compestris*	*Pel A1, Pel A2*	yT&A, pOK12,pRK415	*E. coli* DH5α	40.19	Pel A1 , pI=8.76	[82]
42	*Bacillus sp. N16-5*	*Pel A*	pUC 18, pET28a	*E. coli* DH5α, *E. coli* BL21	35.9	pI=5.87,pH=11.5, OT=50^0C	[63]
43	*Yersinia enterocolitica*	*YeOGL*	pET28a	*E. coli* BL21 pLysS(DE3)	44.2		[86]
44	*Paenibacillus amylolyticus*	*PelA,PelB*	pUC19 derivatives	*E. coli* DH5α			[88]
45	*Bacillus strain*	*Pel22,Pel 66,Pel 90*	pUC18	*E. coli* NM522			[67]
46	*Bacillus subtilis*	*BsPel*	pETsd	*E. coli* BL21 (DE3)			[68]
47	*Caldicellulosiraptor bescii*	*PL3*	pET-45b	*E. coli* BL21(DE 3)	28.9		[87]
48	*Bacillus pumilus DKS1*	*Pel*	pET20b(+)	*E. coli* XL1Blue *E. coli* BL21	35	pH=8.5 , OT=60°C	[69]
49	*Bacillus subtilis WB600*	*Pel*	pWB600, pWB980	*B.subtilis*	44	pH=9, OT=50°C	[137]
50	*Xanthomonas compestris ACCC10048*		pGEM-T-Easy pET-22b	*E. coli* BL21(DE3)		pH=9, OT=30°C Km=4.9g l^{-1} Vmax=18.13 µmol min^{-1}	[85]
51	*Fusarium solani*	*Pel A*		*E. coli* DH5α		pH-=8 OT=30°C	[73]
52	*Fusarium solani*	*Pel B*	pHILD2	*Pichia pastoris*	29	pH=10,OT=30°C, Km=566 µg/ml Vmax=1000 U/mg	[89]
53	*Fusarium solani*	*Pel C*	pHILS1	*Pichia pastoris*	26	pH=9.5,OT=55°C, Km=670 µg/ml Vmax=1100 U/mg	[90]

S. no.	Source	Gene	Vector	Host	Molecular mass of recombinant PL (kDa)	Characteristics of recombinant PL	References
54	*Fusarium solani*	*Pel A*	pHILD2	*Pichia pastoris*	23	pH=10, Km=1 mg ml^{-1} Vmax 500U ml^{-1}	[91]
55	*Colletotrichum gloeosporioides*		pRD091 pCD101	*E. coli* XL1Blue	35.5		[93]
56	*Fusarium oxysporuum f.sp. lycopersici*	*PL1*	pBluescript/KS+				[138]
57	*Colletotrichum gloeosporiodes*	*Pel 1* *Pel2*	pBluescript II KS		Pel1(33.2) Pel2(32.8)	Pel 1(pI=8.41) Pel2(pI=8.29)	[94]
58	*Colletotrichum gloeosporiodes*		pGEM-7Z, pPCPH-1	*C. magna*			[96]
59	*Phytophthora infestans*		Uni-ZapXR	*E. coli* XL1Blue			[97]
60	*Aspergillus nidulans*	*PelA*	pVBSzqx	*Bacillus subtilis*		pH=8.5, OT=50°C Vmax=77 µmol ml^{-1} Km=0.50 mg ml^{-1}	[99]
61	*Fusarium oxysporium f.sp. cubenserace 1*	*PL1*	pPICZaA	*Pichia pastoris*	24	pH=10, OT=50°C	[92]
62	*Phytophthora capsici*	*Pcpel2*	pET, pMAL	*E. coli* BL21(DE3) pLYsS	44	pH=8.5, OT=40°C	[101]
63	*Penicillium occitanis CT1*	*Pal.,1*	pMOSblue λMOS10X	*E. coli* TOP10 *E. coli* ER1647		pH=6 OT=50°C	[103]
64	*Phytophthora capsici*	*Pcpel 1*		*Pichia pastoris*	66	pI= 6.8	[102]
65	*Colletotrichum coccodes*	*Ccpel1*	pGEM-T-Easy pGFP-CA pNV15, poE				[95]

Comparison of 1212 bp long *pel*E and 1173 bp long *pel*D gene of *E. chrysanthemi* B374 shows good homology in the coding region and relatively less homology in 5' and 3'non coding region. The signal sequence in *pel*E and *pel*D contains four and three basic residues which are followed by a stretch of seven and eleven hydrophobic amino acids respectively. The cleavage site for *pel*D signal sequence is ala-ser-ala at position 41 where as in *pel*E cleavage site is asn-arg-ala at position 31.In *pel*E, a potential ribosome binding site AGGAA is positioned 5' of the putative ATG start codon at base 311.At position -102 relative to the ATG start codon there is a TTCACA-(18 bp)-CATAAA sequence which has nine bases conserved out of the 12 bases of the consensus *E. coli* σ^{70}promoter sequence. At position -122 relative to the putative ATG start codon,a sequence highly homologous to the *E. coli* CAP-binding site consensus sequence is present. In case of *pel*D,two putative *E. coli*-like σ^{70} weak promoter sequences are present 65 bp and 33 bp upstream of the putative ATG start codon. Twenty one base pair downstream of the TAA stop codon, an 8bp GC-rich inverted repeat followed by a stretch of 6 Ts is present which serves as a rho-independent terminator sequence [48].

Analysis of PelA and Pel E encoding acidic (pI4.2) and basic (pI 10.0) pectate lyase protein of *Erwinia chrysanthemi* EC16 revealed gene duplication and divergence [47]. In *Erwinia chrysanthemi* 3937, five major isoenzymes of pectate lyase designated as PelA to PelE [50] along with a set of secondary pectate lyases namely PelL [51], PelI [34], PelZ [52] and Pel X [53] have been reported. The ORF of *pelL* gene begins with ATG codon at position 410 and ends with TAA at 1685 position. It codes for 425 amino acid long protein which includes amino terminal signal sequence of 25 amino acids. The start codon is preceded by the potential ribosome binding site GAGG. The potential promoter region shows homology to the classical σ70 promoter. Probable operator region contains two imperfect inverted repeats A1-A2 (AGAGGCTGCG-3nt- CGCAGCCTTT) and B1-B2 (ATGATTTT-3nt-AACATCAT), found at positions 318 and 390 respectively.Two imperfect direct repeats D1-D2 (TGACGACAT-1 nt TGACGAAAT) are also found in this region which overlaps the -35 element of the potential promoter.

A GC-rich inverted repeat (GGCTGC-4nt-GCAGCC) followed by a stretch of T residues is found 32 nucleotides ahead of translation stop codon.This sequence is believed to be involved in termination of *pel*L transcription. The ORF of pelI begins with start codon ATG at position 892 separated by potential ribosome binding site, AAGGAG, and ends with TGA at position 1924.A potential KdgR box, TAAAAAACA GATCTTTGTC is centered 19 nucleotides upstream from the putative *pelI* promoter.This sequence is believed to regulate the transcription of *pel*I gene.However, this sequence differs from the consensus by the absence of a T residue at position 13 which is conserved in all operators that interact with the KdgR protein. A potential CRP-binding site partially overlaps the KdgR binding site (ACAGATCTTTGTCACA) suggesting that *pelI* transcriptionmight be activated by CRP.A GC-rich inverted repeat followed by a stretch of T residues, typical of a Rho-independent transcription terminator is centered 69 nucleotides after the *pelI* translational stop.The ORF of *pelX* begins with ATG codon at position 503 and endswith TAA at position 2705. The *pelX* ATG start codon is preceded by the potential ribosome binding site GGGGAA 3 nt upstream and by a potential promoter 16 nt upstream.A potential KdgR box, with 17 of 18 nt conserved, AAAGAAACANTGTTTCATTis centered 12 nt upstream from the putative *pelX* promoter. A potential CRP-binding site $TGTGAN_6CAAAA$ partially overlaps the KdgR-binding site. The *pelX* translational stop is followed by a GC-rich inverted repeat located 26 nt downstream.

A single copy pectate lyase gene from *Bacillus subtilis* genome was confirmed through southern hybridization and was expressed in *E. coli* [54]. It contains a single open reading frame of 1,420 bp starting with an ATG codon at nucleotide 205 and stops with a TAA termination codon at 1,464 coding for protein of 420 amino acids. There is a typical 21 amino acids signal sequence at the amino terminal.A purine-rich sequence (AGAAAATGGGGGTA)is present upstream of ATG initiation codon and is believed to function as ribosome binding site (RBS). Upstream from the RBS, there are putative -35 (TGAATG) and -10 (TATATT) promoter signals between 121- 126 and between 144- 150 nucleotides, respectively. The sequence at -10 regions is homologous to -10 promoter sequence recognized by the σ^{43}transcription factor of *B. subtilis* and also by the σ^{70}transcription factor of *E. coli.* An inverted sequence is present downstream of TAA stop codon between 1,475 and 1,508 nucleotides which can form secondary structure that may have role in transcription termination.

Two unusual PL showing activity on highly esterified citrus pectin has been cloned which shows similarity with PL of *Fusarium solani, Erwinia carotovora, Erwinia chrysanthemi* and *Bacillus subtilis* yvpA gene [55,56]. The *pel*A gene contains an ORF of 666 nucleotides encoding a protein of 222 residues. The N-terminal signal sequence of 25 residues contains two positively charged amino acids at its N-terminal end, followed by a hydrophobic stretch of 18 amino acids. This hydrophobic stretch is followed by proline and an amino acid region with the sequence AAAA.There is an AAGGGAGGA sequence eight nucleotides upstream of the ATG start codon, resembling that of a ribosome-binding site. Upstream of the *pelA* structural gene, a putative promoter sequence, identical to those recognized by the σ^A subunit of *Bacillus* RNA polymerase, showing a -35 (TAGACA) and -10 (TCAAAT) region is found. In the 3' region of the pectate lyase gene, an inverted repeat of 20 bp, which could act as a transcriptional terminator is present.

The ORF of *pel*C gene encoding protein of 221 amino acids starts with TTG as initiation codon. This start codon is often found in *Bacillus* genes and attaches itself to translation efficiency. There is a GGGGAGGA sequence seven nucleotides upstream of the start codon, resembling that of a ribosome-binding site. In the 3' region of the structural gene, there is an inverted repeat of 14 bp, which could act as a transcriptional terminator. A thermostable and highly alkaline pectate lyase from *Bacillus licheniformis* was also cloned in *E. coli* and was found to be active on citrus pectate as well as sugar beet pectin [57]. The gene has 1026 bp ORF with a putative promoter sequence and a ribosome-binding site (5′-GGAGG-3′) located at distances of 216 bp and 6 bp, respectively, upstream of the ATG start codon.Six nucleotides inverted repeat downstream of the TAA acts as transcriptional termination.

Several alkaline pectate lyase were cloned from different species and strains of *Bacillus* namely *Bacillus* KSM-P103 [58], *Bacillus* sp P-4-N [59], *Bacillus* sp BP 23 [55], *Bacillus* KSM P 15[60, 61], *Bacillus subtilis* IFO3134 [62], *Bacillus* sp N16-5, *Bacillus subtilis* WSH B04-02 [63], *Bacillus* sp N16-5 [64]. These pectate lyase genes contain ORF in the range of 960- 1260 bp with an exception of *pel*-15H gene which has an ORF of 2031 bp encoding mature enzyme of 69.5 kDa. The *pel103* gene has ATG initiation codon at position 190 and TAA stop codon at position 1,225. The sequence from nucleotide 10 to 39 resembles consensus sequence of σ^Atype promoter. The potential -35 region(5'-TTGGGT-3') and -10 region (5'- TGGAAT- 3') are separated by 18 bases.A long inverted repeat extends from nucleotide 1233 to 1269 and is known to be playing significant role in the termination of transcription.

The ORF of *pel-15H* gene starts with initiation codon ATG at position 230 and ends with TAG codon at nucleotide 2261.The putative ribosome binding sequence 5'- AAGGA- 3' is found eighteen bp upstream of initiation codon.The potential -35 sequence (5'- TTGTGG-3') and -10 sequence (5'-TAAATT-3') of the promoter are separated by 17 base pairs. Two palindromic sequences are found 71 and 169 bp downstream of TAG stop codon. Similar in size to the ORF of *pel103* gene, *pel-4A* starts with ATG codon at position 762 and ends with TAA codon at 1799.Eight nucleotides upstream of this ORF is the sequence 5'-AAAGAGGT-3', which is presumably the ribosome-binding site. Separated by 16bp, there is sequence5'-TTGAAT-3'as the potential –35 region and 5'-TATATT-3'as the potential –10 region of σ^A type promoter of *B. subtilis*.A long inverted-repeat sequence is found 57 bp downstream of the termination codon from position 1856 to 1886.The *pel*-15E gene carries a 960 bp ORF starting with ATG initiation codon at position 422 ending with TAG stop codon at 1382.The putative Shine-Dalgarno sequence 5'-AGGAG-3'is found 9 bp upstream of the initiation codon.There is a putative sequence of σ^A-type promoter of *B. subtilis*, with 5'-TAGACA-3' and 5'-TATACT-3'located 235 and 210 regions respectively and separated by 17 bp. A palindromic sequence is located 18 bp downstream of TAG termination codon.

Pectate lyase genes from *Bacillus subtilis* expressed in *Pichia pastoris* was found to be active 10 times higher than when expressed in *E. coli* [65,66]. Several other PLs designated as *pel22*, *pel66* and *pel90* from *Bacillus* strain [67], BsPel from *Bacillus subtilis* [68], *Bacillus pumilus* DKS1 [69], *Bacillus subtilis* WB600 [70] have been cloned in *E. coli*. The ORF of *pel66* and *pel90* is 1260 bp in length while that of *pel22* the ORF is 1062 bp in length. The ORF of *pel22*, *pel66* and *pel90* starts with ATG at positions 142, 402 and 152 respectively. Termination codon for *pel66*, *pel90* is TAA at positions1664 and 1414 while stop codon for *pel22* is TGA at position 1206. The PL gene *Apel* from *B. subtilis* carries an ORF of 1,260 bp, encoding a signal peptide of

21 amino acids and a mature protein of 399 amino acids.

Besides *Erwinia* and *Bacillus* genus, pectate lyase genes have been cloned from other bacterial sources. A PLY gene from *Yersinia pseudotuberculosis* has been cloned and expressed in *E. coli* [71]. The open reading frame of *pely* gene spans 1623 bp. The start codon ATG is preceded by purine rich sequence which functions as ribosome binding site.A pel gene from *Pseudomonas viridiflava* [72] and *Pseudomonas fluorescens* was cloned and expressed in *E. coli* strain not being influenced by carbon source or Ca^{++} [73]. A Pel gene from *Pseudomonas marginalis* N6301 [74] has an open reading frame which begins with an ATG codon at position 517 and ends with a TAA codon at position 1657.A potential Shine-Dalgarno sequence is present in the 5' region of the putative ATG start codon. *E. coli* σ^{70} like promoter sequences are found in 5' region of ribosome binding site.Inverted repeat sequences are present at positions 410-434 and 460-484 which may be involved in regulation of gene expression.

The ORF of *pelS* from *Pseudomonas syringae* pv *lachrymans* encodes for 40.3 kDa protein including signal sequence of 29 amino acids. The ribosome-binding site is located 10 bp upstream of the start of the *pelS* ORF. A potential σ^{54}promoter is predicted between 43 and 60 bp upstream of the start site. An interesting feature of *pelS*gene is that the inverted repeats downstream of the gene are not followed by T-rich region typical of rho-independent terminators [75]. A Pel 10 A from *Pseudomonas cellulosa* [76] and 2 genes namely Pel A and Pel B from *Pseudoalteromonas haloplanktis* ANT1505 [77] were cloned and expressed in *E. coli* strains. The *pel-10A* gene contains a long ORF of 1950 bp which codes for protein of molecular mass 68.5 kDa. The ribosome binding sequence 5'-AAGGA-3' is similar to the ribosome binding sequence of Gram-negative bacteria. Sequence analysis of the predicted amino acid sequence of Pel10A revealed a typical Gram-negative signal peptide of 31 residues in length and three stretches separated by two serine-rich linker sequences.

A pectate lyase gene has been isolated from *Amycolata* sp by the activity screening of a genomic DNA library in *Streptomyces lividans* TK24 [78]. It has 930 bp ORF having the initiation codon ATG at position 316 and terminating with TGA at position 1246. It codes for a protein of 310 amino acids having a putative leader sequence of 26 amino acids.The protein does not contain cysteine. A leader peptidase cleaves after the sequence Ala–Thr–Ala. The leader sequence contains a positively charged N-terminal followed by a hydrophobic domain and a proline residue.Seven bp upstream of start codon, the potential Shine- Dalgarno sequence 5'-GGGAG-3' is present. A short inverted repeat of 7 bp follows the 3' end of the *pel* gene, which could function as a transcriptional terminator.

The pelA gene from *Azospirillum irakense*, a N_2-fixing plant-associatedbacterium,has been isolated by heterologous expression in *Escherichia coli* [79]. It has GTG initiation codon. A potential Shine-Dalgarno sequence GAGGAA is located12 bases upstream of the start codon.The signal sequence has positively charged amino terminus, a hydrophobic core of 12 residues in the center, and two alanine residues at positions 21 and 23 relative to the processing site. A signal peptidase I cleavage site is located between amino acids 24 and 25.

The pelA gene from *Treponema pectinovorum* ATCC 33768, which is an oral spirochete, was isolated by heterologous expression of a cosmid library in *E. coli* [80]. It has an open reading frame of 1293 bp which codes for a protein of 430 amino acids. A putative N-terminal signal sequences of 21 amino acids produces a mature protein of 46.7 kDa when cleaved. Pel A gene was cloned from a hypothermophilic bacterial strain *Thermotoga maritima* and expressed in *E. coli*. The recombinant pectate lyase was found to be thermostable, operating optimally at 90^0 C and pH 9.0. Half- life of this enzyme for thermal inactivation was almost 2 h at 95^0 C [81].

Two pectate lyase genes *pel*A1 and *pel*A2 from *Xanthomonas campestris pv. campestris* (Xcc) causing black rot in crucifers have also been cloned. It was found that *pel*A1 codes for the major pectate lyase in Xcc strain Xc17 and its expression is up-regulated by cAMP receptor protein-like protein (Clp). RpfF, an enoyl-CoA hydratase homologue, positively regulate *pel*A1 transcription [82]. A cold-active pectate lyase gene cloned from *Xanthomonas campestris* pv. *campestris* was heterologously expressed in *E. coli* and the recombinant protein was purified and biochemically characterized [83].

A single beneficial mutation R236F in pectate lyase from *Xanthomonas campestris* (PLXc) resulted a 23-fold increase in the half-life at 45°C

and a 6°C increase in Tm without altering the catalytic efficiency of the enzyme by a strategy based on Melting-Temperature-Guided Sequence Alignment [84]. An alkaline pectate lyase (PL D) has been cloned from *Xanthomonas campestris* ATCC 10048 and the recombinant protein PLD (r-PL D) produced in *Escherichia coli* was purified showing higher activity over a wide pH and at lower temperatures [85]. A *Ye*OGL, Oligogalacturonate lyases OGLs from *Yersinia enterocolitica,* also classified as pectate lyase family 22 has been cloned and expressed [86]. X-ray structure of YeOGL reveals the presence of Mn^{2+} ion in the active site which is co-ordinated by three histidine, one glutamine and one acetate ion.Histidine, a residue that is highly conserved throughout the OGL family, abstracts the α-proton in the -1 subsite and represents a unique catalytic base among pectate lyases.Cloning and expression of PL3 from *Caldicellulosiraptor bescii* revealing structure and mode of action of pectate lyase has been reported recently [87]. Two pectate lyase genes Pel A and Pel B were cloned and characterized from *Paenibacillus amylolyticus.* These Pel A and Pel B enzymes show an unusual combination of pectate lyase and pectin lyase activity by degrading both polygalacturonic acid and highly methylated pectin, respectively [88].

5.2.2 Fungal pectate lyases

As compared to bacterial sources there are only few reports of gene cloning and expression studies of fungal pectate lyases. Pectate lyase genes namely *pel* A, *pel* B and *pel* C have been cloned from *Fusarium solani* f. sp. *pisi* (*Nectria haematococca* Mating Type VI) and expressed in *Pichia pastoris* [89-91].The nucleotide sequence of *pelB* shows a single open reading frame of 732 bp interrupted by two introns of 72 and 50 bp and coding protein of 244 residues. The signal peptide of 16 amino acids has cleavage site between ala-16 and ala-17, resulting in a mature protein of calculated molecular mass 24.2 kDa. One TATAAAA box is found 107 bp upstream of the ATG start codon. A CAAT motif is predicted 33 nucleotides upstream from the predicted TATA box. The sequence ATAAAA is found 206 nucleotides downstream from the stop codon. Similar to *pelB* the open reading frame of *pelC* carries two introns of 56 and 51 bp. However no signal peptide is present at the N-terminal of the protein. Similar to the fungal TATAAA box involved in transcription, a TATATAA box was found 162 bp 5' to the ATG start codon. Instead of polyadenylation sequence, a similar stretch of AACAAA is present 175 bp downstream of termination codon.

Similary *PL*1 from *Fusarium oxysporium f.sp. cubenserace* has also been expressed in *Pichia pastoris* [92]. Pectate lyase from *Colletotrichum gloeosporioides* [93,94] and *Colletotrichum coccodes* Ccpel [95] have also been cloned. The *pel1* and *pel2* gene from *C. gloeosporioides* revealed the presence of ORF of 1002 bp and 990 bp respectively, both being interrupted by introns. The polyadenylation sequence AATAAA and the site for the addition of the poly(A)+ in *pel1* is located 149 bp and 173 bp downstream of the stop codon, respectively. In *pel2*, the polyadenylation sequence of AATAAA is absent and the site for the addition of the poly(A)+ tailis located 88 bp downstream from the stop codon. The expression of pectate lyase genes from *Colletotrichumgloeosporioides* in *Colletotrichum magna* reveals the possible role of pectate lyase in pathogenesis [96].

A pectate lyase like gene *mpl1* cloned using the cDNA-representational difference analysis subtraction method was found to be activated during mating of A1 and A2 strain of *Phytophthora infestans*. It was found to contain a continuous open reading of 789 bp. More than 13 genes with sequences similar to that of *mpl1* have been found in the genome, indicating *mpl1* to be a multicopy gene [97]. Twenty two full length pectate lyase genes were from *Phytophthora capsici* [98]. Of these, 12 pectate lyase genes were found to be highly induced during infection of pepper. Pel A gene from *Aspergillus nidulans* has been successfully expressed in *Bacillus subtilis* [99] and *E. coli* [100]. Pectate lyase gene designated as *Pcpel2* and *Pcpel1*from *Phytophthora capsicin* have been cloned and expressed in *E. coli* and *Pichia pastoris* respectively [101,102] revealing its significant role in pathogenesis. The open reading frame of *Pcpel1I* gene is 1233 bp and encodes 410 amino acid polypeptide, including 21 residues long amino terminal signal sequence. There are 6 putative N-glycosylationsites (N66, N77, N123, N139, N317 and N372), without any intron.The CT1 mutant of *Penicillium occitanis* is known to hyper produce extracellular pectinases and the fragments including a pectate lyase (*pal1)* has been isolated using cDNA and RT PCR. It has been observed that the CT1 mutation affects a trans-regulatory transcriptional factor influencing pectinase expression [103].

6. CONCLUSION

Pectate lyases are an important member of pectinases which are considered to be one of the virulence factors for pathogenesis and one of the major causes of fruit ripening. The potential application of microbial pectate lyases in the textile industry for more economical and eco-friendly approach demands its large scale production. Besides searching for the novel microbial sources, its production optimization by solid state and submerged fermentation processes, substantial efforts have been made to clone and over-express the relevant pectate lyases genes. The directed evolution approach for novel pectate lyase for enzymatic scouring of cotton fabric has also been attempted. An insight to diversity of pectate lyase genes from diverse microbial sources is highlighted in this review.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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