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Computational Insight into PCR Amplified Pectate Lyase Genes from Different Species of *Aspergillus*

Manish Kumar[1], Jeya Nasim[1], Sangeeta Yadav[1], Aiman Tanveer[1] and Dinesh Yadav[1*]

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

Authors' contributions

This work was carried out in collaboration between all authors. Author MK performed the wet lab experiments and some bioinformatics work. Author JN carried out the bioinformatics work. Authors SY and AT analyzed the results obtained, prepared suitable tables and figures and made the first draft of the manuscript. Author DY conceived the idea, made the work plan and corrected the final draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Pectate lyase (PL) is an important member of pectinase group of enzyme associated with cleaving α -1, 4 linkages in pectate polymers by a β-elimination mechanism, producing 4, 5–unsaturated oligogalacturonates. In the present study, PCR amplification of pectate lyase genes from different species of *Aspergillus* (namely *A. oryzae, A. flavus*, *A. terreus*, *A. fumigatus* and *A. niger*) was attempted. The expected size bands resulting from amplification using different sets of primers were gel eluted and sequenced using gene specific primers. The sequences of putative 23 pectate lyase (*PL)* genes subjected to BLAST analysis revealed its identity to pectate lyase sequences available in databases. Ten sequences of PCR amplified *PL* genes of *A. oryzae* namely PL-001 to PL-010 representing five identified *PL* genes from two different strains revealed maximum identity with the available sequences of *A. oryzae* RIB40 pectate lyases, *A. flavus* NRRL 3357 in the range of 98-100%. The phylogenetic tree constructed based on protein sequence of putative *PL* genes

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

representing different strains of *A. oryzae, A. terreus, A. flavus, A. fumigatus* revealed several clusters and sub-clusters. A total of 12 *PL* gene sequences representing *A. oryzae* MTCC 3782, *A. oryzae* MTCC 6993, *A. fumigatus* MTCC 3070, *A. fumigatus* MTCC 2584 and *Aspergillus flavus* MTCC 8835 were submitted to GenBank and were assigned accession numbers KP869835 to KP869846.

Keywords: Pectate lyase; PCR amplification; multiple sequence alignment; motif; BLAST; Aspergillus.

1. INTRODUCTION

Pectinases constitute a complex group of enzymes associated with degradation of various pectic substances (pectin) present in the middle lamella of plant cell wall and have been classified according to their mode of action and substrates [1]. Important members include pectin esterases [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]. The importance of pectinases is evident from the fact that its production represents about 10% of the overall manufacturing of enzyme preparations [2]. These groups of enzymes have been reviewed extensively in light of their potential industrial applications such as clarification of fruit juices, retting of natural fibers, treatment of pectic waste water, coffee and tea leaf fermentation, oil extraction, virus purifications and developing functional foods [2-15].

Pectate lyase (PL) is capable of cleaving α -1,4 linkages in pectate polymers by a β-elimination mechanism, producing 4,5–unsaturated oligogalacturonates [16]. Depending on its mode of action pectate lyase can be categorized as endo PL (4.2.2.1) and exo PL (4.2.2.2). PL has an absolute requirement of Ca^{2+} ions hence it is strongly inhibited by chelating agents such as EDTA [8]. The β-elimination mechanism is possible only when the polysaccharide contains uronic acid residues like galacturonate, glucuronate, iduronate, mannuronate or guluronate. Pectin lyase also shows β-elimination mechanism but it only attacks highly methyl-esterified regions while pectate lyases prefer chains with a low degree of methyl-esterification [11]. 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 [10].

Pectate lyase like (PLL) family genes have been extensively explored in *Erwinia chrysanthemi* [17]. A total of seven putative *PL* clones were screened from the genomic library of *Erwinia chrysanthe*mi EC16 [18]. In *Erwinia chrysanthemis* 3937, five major isoenzymes of pectate lyase designated as PelA to PelE [19] along with a set of secondary pectate lyases namely PelL [20], PelI [21], PelZ [22] and Pel X [23] has been reported. PLL genes have also been studied in tomato and two genes Anther Tomato 56 (*LAT*56) and *LAT*59 revealed strong sequence homology with the PelC of bacterial PL [24]. The existence of pectin lyases in multiple copies mainly due to gene duplication events has been reported [25]. In *Arabidopsis* there are 26 pectate lyase like genes, while rice has only 12 gene copies [26,27]. This might be due to multiple gene duplication events.

The microbial pectate lyases are preferred for the bioscouring applications and directed evolution of a novel pectate lyase for processing cotton fabric has been attempted [28].The microbial alkaline pectate lyases have applications in several fields like paper making, coffee and tea fermentation, textile and fiber processing, oil extraction and wastewater treatment [7,29].

Several pectate lyase genes, predominately from bacterial sources, have been cloned and expressed in different host cells. The cloning and expression studies of pectate lyases genes have been extensively reported in bacteria *Erwinia chrysanthemi* and *Erwinia caratovora*. 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 [30]. Recently cloning and expression of pectate lyase genes from *Paenibacillus* sp. 0602 in *E. coli* has been reported [31].

Pectate lyases have also been annotated in few of the *Aspergillus* species especially *A. nidulans, A. clavatus, A. oryzae and A. niger* revealing existence of multigene families with *A. oryzae* having maximum number of genes [32].

Computational studies on pectin lyases protein sequences for homology search, multiple sequence alignment, phylogenetic tree construction and motif analysis using different bioinformatics tools have been attempted [11].

PCR amplification and sequence analysis of pectin lyase genes [33] along with *in-silico* characterization of polygalacturonases protein sequences have been reported [34]. Computational assessment of predicted three dimensional structures of pectate lyases from different species of *Aspergillus* using homology modelling has been attempted recently [35].

This paper reports PCR amplification of pectate lyase genes from different *Aspergillus species*, sequencing and *in silico* sequence analysis for homology search, multiple sequence alignment, phylogenetic tree construction and motif search.

2. MATERIALS AND METHODS

2.1 Fungal Strains and Culture Conditions

The fungal strains were procured from culture collection centers (namely Microbial Type Culture Collection & Gene Bank (MTCC), Institute of Microbial Technology (CSIR) Chandigarh, India and National institute of Interdisciplinary Culture Collection (NIICC) National institute of Interdisciplinary Science and Technology, Thiruvananthapuram, Kerala, India). The cultures were grown on CYE (Czapek Yeast Extract) agar plates for 3 days at 26- 30℃. The fungal spores were then inoculated in CYE broth and Kept at 26- 30℃ at 160 rpm for 5 days. The mycelia grown were filtered, dried, and used for DNA extraction.

2.2 Genomic DNA Isolation

Genomic DNA was isolated from the mycelia of different *Aspergillus* strains by a standard method [36] using a slightly modified extraction buffers containing 5% SDS, 50 mM Tris (PH 8), 50 mM EDTA (PH 8), 100 mM NaCl, 1% β mercaptoethanol and 100 µg/ml Proteinase K. The quality and quantity of genomic DNA were analyzed by standard methods using agarose gel electrophoresis and UV-Vis spectrophotometry [37].

2.3 Primer Design

Available nucleotide sequences of pectate lyase genes from different species of *Aspergillus* (*Aspergillus flavus, A. oryazae, A. fumigatus, A. niger, A. terreus, A. clavatus. A. niulans*) were retrieved from GenBank database of NCBI. These sequences were used to design primers using software DNA star and Primer 3 (http://biotools.umassmed.edu/bioapps/primer3).

All the pectate lyases reported in this study contained introns. The primers were designed against the ORF of pectate lyase genes. The primers were further checked by Primer BLAST and were synthesized commercially by Merck Biosciences, India. The list of primers used for PCR amplification of pectate lyase genes is shown in Table 1.

2.4 PCR Amplification and Gel Elution of Expected Size Amplicons

For PCR amplification of pectate lyase genes approximately 100 ng of template genomic DNA, 30 ng of primers, 1.5 units of *Taq* DNA polymerase, 1.5 mM $MgCl_2$ were used for each 25 µl reaction. The PCR conditions of initial denaturation were 95℃ for 5 min, followed by 35 cycles of repeated three steps comprising denaturation at 94℃ for 1 min, annealing conditions were variable based on Tm values of respective primers while extension was performed at 72℃ for 2 min. After completion of 35 cycles, a final extension of 72℃ for 5 min was used and then the PCR amplicons were analyzed on 1.5% agarose gel. The expected size amplicons were eluted from agarose gel using Hipura A-Gel elution kit (Himedia) and Gene JET Gel Elution kit, (Thermo Fischer scientific) as per the manufacturer's instructions, quantified and sequenced using gene specific primers commercially.

2.5 *In silico* Analysis of Pectate Lyase Sequences

The sequences of pectate lyases were subjected to homology search with NCBI Database using BLAST program [38]. Multiple sequence alignment of the sequences and construction of phylogenetic tree were performed by Clustal X2 through MEGA 5 [39] by Maximum Likelihood method. Multiple sequence alignment gives an indication about the conserved residues present in the protein sequences. The deduced protein sequences of the pectate lyase were analysed by MEME (http://meme.sdsc.edu/meme/meme.html) [40] for identification of conserved motifs.

3. RESULTS AND DISCUSSION

3.1 PCR Amplification of Pectate Lyase Genes

PCR amplification of pectate lyase (*PL*) genes using template DNA from different species of *Aspergillus* namely *A. oryzae* (strains MTCC

3782, MTCC 6993), *A. flavus* (strains NIICC08142, NIICC08147, MTCC8835), *A. terreus* (strains MTCC 2580, MTCC3006), *A. fumigatus* (strains MTCC 2584, MTCC 3070) and *A. niger* MTCC 478 with different sets of primers was performed. The PCR amplification of *PL* genes for *Aspergillus oryzae* MTCC 3782 using five sets of primers (*i.e.* PL14, PL15, Pl16, PL17 and PL31) resulted in products of approximately 1439 bp, 941 bp, 975 bp, 1413 bp, and 944 bp respectively (Fig. 1).

The PCR condition standardized comprised of a single step of initial denaturation at 95℃ for 5 minutes, followed by repeated 35 cycles of denaturation at 94℃ for 1 minute, annealing at 44.5-47.5℃ for 1 minute (47.5℃ for PL14, PL-15 and PL-16; 44.5℃ for PL17 and 46.5℃ for PL31), extension at 72℃ for 2 minutes followed by a final extension at 72℃ for 5 minutes. A distinct single band of expected size was obtained with all the primer pairs. Similarly using the same sets of primers all the five *PL* genes were PCR amplified with another strain of *A. oryzae* i.e. MTCC 6993, subjected to the same PCR conditions (Fig. 1).

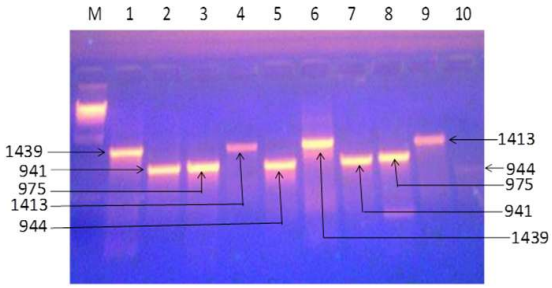

Fig. 1. Agarose gel (1.5%) showing eluted PCR amplified pectate lyase genes of *Aspergillus Oryzae* MTCC 3782 and *Aspergillus Oryzae* MTCC 6993 with primers GCEL-PL14, 15, 16, 17& 31 respectively (Lanes 1-10); Lane M-Lambda *Hin*dIII marker DNA

PCR amplification of *PL* genes with primers PL23, PL24, PL28, PL29, PL32 and PL 33 using different strains of *Aspergillus flavus* revealed similar pattern. For *A. flavus* NIICC 08147 template DNA, primers PL23, PL24, PL28, PL29, PL32 and PL33 resulted in expected size product *i.e* 1398 bp, 1556 bp, 846 bp, 1626 bp and 1079bp respectively, though primer dimers were also observed (Fig. 2). The annealing temperature standardized for PCR amplification with primers PL23, PL24, PL28, PL29, PL32 and PL 33 were 48.5, 44.5, 45.5, 46.5, 46.5 and 47.5ºC respectively. Similarly expected size bands were obtained by PCR amplification using same sets of primers for another strain of *A. flavus* NIICC08142 and *A. flavus* MTCC 8835.

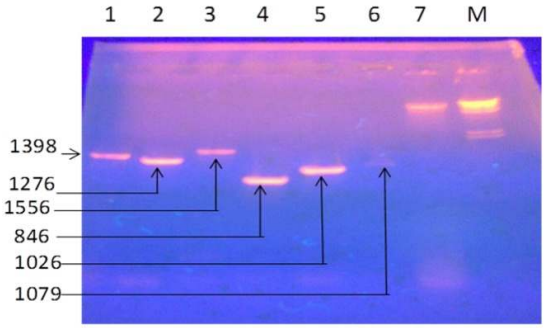

Fig. 2. Agarose gel (1.5%) showing PCR amplified pectate lyase genes of *Aspergillus flavus* NIICC 08147 with primers GCEL-PL23, 24, 28,29, 32 & 33 respectively (Lanes 1-6); Lane 7, genomic DNA of *Aspergillus flavus* NIICC 08147; Lane M-Lambda *Hin*dIII marker DNA

With template DNA of *A. flavus* MTCC 8835, all the six *PL* genes were amplified under similar PCR conditions resulting into expected size bands, though with primer PL29 two bands were observed. The PCR amplification, sequencing and bioinformatics analysis of seven pectin lyase genes from different strains of *A. flavus* has been reported elsewhere [41] and the sizes are mentioned in the result section.

The PCR amplification of four *PL* genes using gene specific primers namely PL18, PL19, PL20 and PL25 for *A. fumigatus* was attempted at different annealing temperatures *i.e.* 45.5℃, 47.5℃, 47.5℃ and 45.5℃ respectively. The template DNA of two strains of *A. fumigatus i.e.* MTCC2584 and MTCC3070 was used for PCR amplification resulting in the apparent size bands of 808bp, 803bp, 1170bp and 1330bp with primers PL18, PL19, PL20 and PL25 respectively. The *PL* genes were also PCR amplified from two strains namely *A. terreus* MTCC2580 and *A. terreus* MTCC3006, resulting in the expected size bands of 818bp and 1140 bp with primers PL26 and PL27 respectively. Similarly *PL* gene from *Aspergillus niger* MTCC478 was PCR amplified with primer PL 30 under similar PCR conditions at 50°C annealing temperature resulting in expected size band of 1180 bp. The expected size bands resulting from different sets of primers for different template DNAs were gel eluted and sequenced using gene specific primers.

3.2 Sequence Analysis of PCR Amplified Pectate Lyase Genes

A total of 23 putative pectate lyase genes designated as PL001 to PL023 amplified from different fungi using gene specific primers were sequenced with both forward and reverse gene specific primers. A total of only 12 sequences representing putative pectate lyase genes of different species of *Aspergillus namely A. oryzae*, *A. fumigatus, A. flavus, A. niger* and *A terreus* showed good read length and were submitted to GenBank for accession numbers (Table 2). Out of 12 pectate lyase gene sequences, two of them namely PL016 (KP869836) and PL020 (KP869846) could not be verified. The sequencing of PCR amplified putative pectate lyase genes using gene specific primers resulted in the variability in the sequence length, which could be minimized by sequencing using universal primers, once cloned in appropriate vector. The sequence of PCR amplified pectate lyase genes with the assigned accession number is shown in Table 2.

The BLAST analysis of these nucleotide sequences revealed its identity to pectate lyase sequences available in databases. The two sequences showing maximum similarity with the sequenced pectate lyase genes, along with a maximum score, total score, query coverage, E-value and maximum identity were analysed. Ten sequences of PCR amplified pectate lyase genes of *A. oryzae* representing five identified *PL* genes from two different strains revealed maximum identity with the available sequences of *A. oryzae* RIB40 pectate lyases, *A. flavus* NRRL 3357 in the range of 98-100%.

Similarly sequences of three *PL* genes of *A. fumigatus* from two strains namely MTCC 2584 and MTCC 3070 showed maximum similarity with *A. fumigatus Af 293* in databases when subjected to BLAST. A report of pectinases genes of *A. fumigatus* is also available [42]. A putative pectate lyase gene sequence obtained from PCR amplified product using two strains of *Aspergillus terreus* namely MTCC3006 & MTCC2580 revealed its identity with *A. terreus* NIH 2624 (accession number XM001217211.1) under BLAST analysis. A putative PL gene sequence of *Aspergillus niger* MTCC 478 showed 91% maximum identity with *A. niger* pectate lyase A (Accession number AJ276331.1). Several pectin lyase genes from *Aspergillus niger* have been reported [43,44,45, 46]. Expression profiling of pectinolytic genes of *A. niger* was also performed by de Vries et al. [47]. Expression profiling of pectinolytic genes of *A. niger* has also been attempted and its regulation has been investigated [48,49]. Similarly two *PL* genes of *Aspergillus flavus* showed 98% maximum identity with *A. flavus* NRRL 3357 pectate lyase A genes (accession numbers XM002378480.1; XM002377918.1). Sequence analysis of PCR amplified seven pectin lyase genes from *A. flavus* NIICC8142 has recently been reported [41].

A total of 10 pectate lyase gene sequences having assigned GenBank accession numbers representing different species of *Aspergillus* namely *A. oryzae, A. fumigatus, A. terreus, A. niger* and *A. flavus* were subjected to multiple sequence alignment after translation to respective pectate lyase proteins. These protein sequences revealed several conserved amino acid residues (Fig. 3). Due to the partial sequences of the pectate lyase genes, the translated proteins could not be extensively analysed for the presence of conserved amino acid residues.

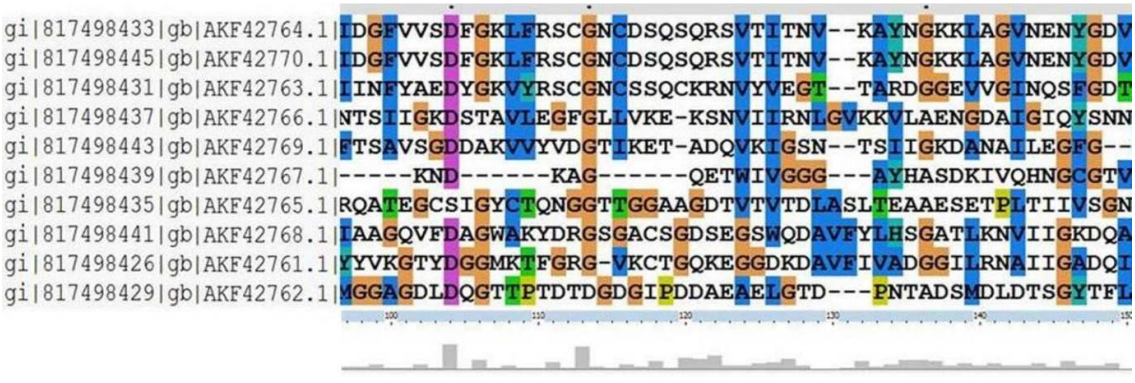

Fig. 3. Multiple sequence alignment of 10 pectate lyase protein sequence revealing conserved residues

Table 1. List of primers designed for PCR amplification of pectate lyase genes from different species and strains of *Aspergillus*

Primer code	Source and accession number	Primer (F on top and R at bottom)	Base count	Tm	G+C %	Product length
PL014	*Aspergillus oryzae*	GCAAGGCATGATGAACACCC	20	59.83	55.00	1450 bp
	XM_001822263.1	CAATATGCGGCTCACTCCCT	20	59.83	55.00	
PL015	*Aspergillus oryzae*	AGTCTTCCAGACAAGCAGAGC	21	60.00	52.38	846 bp
	XM_001825367.2	TTCCCTGATTTCCCACCACC	20	59.00	55.00	
PL016	*Aspergillus oryzae*	GCACACAATGCAGACGCTTG	20	61.00	55.00	812 bp
	XM_001825051.2	ACTCCAGCAATCAGGTGTGG	20	59.96	55.00	
PL017	*Aspergillus oryzae*	GCTCAAGGGAGCACTTTCAC	20	59.00	55.00	1215 bp
	XM_001826216.2	TTCGCGAAGAGAAAAACAAAAGAT	24	58.00	33.00	
PL018	*Aspergillus fumigates*	CCTGACGCGTGTTTGACAAT	20	59.41	50.00	808 bp
	XM_744769.1	TTCCTGTCGCTTTGGCATGT	20	60.54	50.00	
PL019	*Aspergillus fumigates*	CAGCTTTCAGTACAAATAGCCCAT	24	59.36	41.67	803 bp
	XM_743777,1	CAGTTCCCCATCCCCGACTC	20	61.97	65.00	
PL 020	*Aspergillus fumigates*	GGCAGACGAAGGAGTGAAAGA	21	60.00	47.62	1170 bp
	XM_749043.1	CTTCGCTCGTCAAGTCTGGTA	21	59.80	47.62	
PL021	*Aspergillus clavatus*	TCCTTCACAACATCACCCCAA	21	59.50	47.62	830 bp
	XM_001276683.1	CCTCTCCAGCTCTCCGTCTA	20	59.82	60.00	
PL022	*Aspergillus clavatus*	AACATAGGCCAAAAGGCCGTC	21	61.23	52.38	1390 bp
	XM_001273146.1	GCAGAACCACGGAATTACTTCATC	24	60.00	45.83	
PL023	*Aspergillus flavus*	CCAGCAAAGCTGTTCCATTCAC	22	60.61	50.00	1230 bp
	XM_2378480.1	GATCCTGGCCCAGCCAAACA	20	62.79	60.00	
PL024	*Aspergillus flavus*	ACAATTCACAGTTCCCAAGAGC	22	59.11	45.00	1090 bp
	XM_002377918	AAACACTACTAGCGCTTCGC	20	58.65	50.00	
PL025	*Aspergillus fumigates*	TGTGCTTTGCTGTGTCGAAG	20	59.34	50.00	1330 bp
	XM_759043.1	TGGTCAAGCTAAAGCAAGTGGA	22	60.16	45.00	
PL026	*Aspergillus terreus*	CATGTGGTGATCGGCATGAAC	21	59.94	52.00	818 bp
	XM_001217211.1	CATGAGGCCCGTTGCATTCT	20	61.03	55.00	
PL027	*Aspergillus terreus*	TCTGCCTGCCTCGACTTCAA	20	61.47	55.00	1140 bp
	XM_001216744.1	TTACGAGAGATCGCTGCCCA	20	61.00	55.00	
PL028	*Aspergillus flavu*	CTTGTCGATGAATGCCGGAAG	21	59.67	52.38	1400 bp
	XM_002384331.1	ACGATACCGACCAAGAGACAA	21	58.00	47.62	
PL029	*Aspergillus flavus*	ACACTCTGGGAGTGATCCTTTC	22	59.43	50.00	781 bp
	XM_002372580.1	TGAGTATGCGCTTAGCAGGC	20	60.53	55.00	
PL030	*Aspergillus niger*	GCCTCTCACATCGTCTTTCCT	21	59.00	52.00	1180 bp
	XM_001402441.1	GCTGTCCTGCAGATACCAAGA	21	59.00	52.00	

Primer code	Source and accession number	Primer (F on top and R at bottom)	Base count	Tm	G+C %	Product length
PL031	*Aspergillus oryzae*	ATCCATTGACTTCTCGTGCCA	21	59.00	47.62	781 bp
	XM_001817480.1	TGCCTTGCAGGGAGACAAATA	21	59.00	47.62	
PL032	*Aspergillus flavus*	ACCCTCCTCAGTCTTCCAGA	20	59.00	55.00	846 bp
	XM_002384863.1	GTTCTAACTAAAAGCGAGTGAATGA	25	57.00	36.00	
PL033	*Aspergillus flavus*	ACATATGTACACCTCACGCACAT	23	60.12	43.48	914 bp
	XM_002380564.1	GGTGGCTTCAGGTTGAGACA	20	59.89	55.00	

Table 2. List of pectate lyases gene sequences amplified from different species and strains of *Aspergillus*

Sl. No.	PL gene	GenBank accession number	Product size (bp)	Protein ID	Template /Source
1	PL001	KP869835	433	AKF42761	*Aspergillus oryzae* MTCC 3782
2	PL004	KP869837	535	AKF42762	*Aspergillus oryzae* MTCC 3782
3	PL005	KP869838	167	AKF42763	*Aspergillus oryzae* MTCC 6993
4	PL007	KP869840	193	AKF42765	*Aspergillus oryzae* MTCC 6993
5	PL008	KP869839	310	AKF42764	*Aspergillus oryzae* MTCC 6993
6	PL009	KP869842	365	AKF42767	*Aspergillus oryzae* MTCC 6993
7	PL010	KP869843	334	AKF42768	*Aspergillus fumigatus* MTCC 2584
8	PL015	KP869845	328	AKF42770	*Aspergillus fumigatus* MTCC 2584
9	PL016	KP869836	169	Unverified	*Aspergillus terreus* MTCC 3006
10	PL017	KP869841	402	AKF42766	*Aspergillus fumigatus* MTCC 2580
11	PL018	KP869844	211	AKF42769	*Aspergillus niger* MTCC 478
12	PL020	KP869846	600	Unverified	*Aspergillus flavus* MTCC 8835

Table 3. The five conserved motifs observed among translated proteins of sequenced pectate lyases genes with best possible amino acid sequences, frequency of occurrence and conserved domains

Motif no.	Sequence length	Sequence	Occurrence at different site	Conserved domain
1	50	AQGADDKVIQHNSCGEVNIQNFYVQDFGKLYRSCGNCDSQFKRTVQISNV	09	Pectate lyase
2	50	EGGWKDAVFYLHEGATLKNAIIGADQIEGVHCEGACTIEFVWWEEVCEDA	08	Pectate lyase
3	20	ARNGKKLVGINPNYGDTATI	11	Pectate lyase
4	21	EIAAGETFDGGMKTYGRGVEC	08	Pectate lyase
5	21	MLRERSNFPMPNLKITQTFAE	10	Pectate lyase

A phylogenetic tree was constructed using the partial nucleotide sequences representing putative pectate lyase genes of different *Aspergillus* species. The putative pectate lyase genes representing different strains of *A. oryzae, A. terreus, A. flavus* were closely placed in the tree though it was not strictly followed, may be due to lack of complete sequences and it would be more authentic once the translated proteins of these gene sequences are subjected to phylogenetic tree construction. The identified pectate lyases showed close resemblance with the pectin lyases from different *Aspergillus* species.

With the available partial nucleotide sequences of putative pectate lyase genes, attempt was made to translate into protein sequences by subjecting it to *in silico* translation tool. A total of 12 sequences (PL-001, PL-002, PL-003, PL-005, PL-007, PL-008, PL-009, PL-012, PL-016, PL-017, PL-020, PL-021) with comparatively good read length were translated and were assigned GenBank Accession number KP869835 to KP869846. The phylogenetic tree constructed based on translated amino acid sequences of PCR amplified pectate lyase genes along with available pectin lyase protein sequences representing different species of *Aspergillus* revealed two distinct major clusters designated as Group-A and Group-B (Fig. 4).

These major clusters were further bifurcated into two distinct sub-clusters each designated as cluster-I and cluster-II. The *PL* genes of *A. oryzae and A. niger* were closely associated with Group-B along with pectin lyase genes of *A. niger and A. flavus*. Four of the identified pectate lyases were associated with group A which showed close association with pectin lyases of different *Aspergillus* strains (Fig. 4). In general the pectate lyase genes of a particular species of *Aspergillus* were closely placed revealing the sequence level similarity like multiple accessions of pectate lyases of *A. oryzae, A. terreus, A. niger* and *A. flavus.* Pectate lyases and pectin lyases have conserved domain Pec_Lyase_C representing same group of trans-eliminases [1,11] and hence were considered for phylogenetic tree construction. The members of pectin lyases and pectate lyases from different species of *Aspergillus* were placed closely in the phylogenetic tree though few of them were intermingled (Fig. 4). The sequenced pectate lyase with accession number AKF42766.1 was paralogous to AEM66392 of *A. ficum.* Three of the pectate lyase sequences were clustered and formed close grouping with one another in group A. AKF42770.1 of *A. favus* was orthologous to *A. oryzae* pectate lyases AKF42764.1 and AKF42763.1.

The pectate lyase genes with assigned GenBank accession numbers were subjected to motif analysis and distribution of five conserved motifs were assessed (Fig. 5). The pectate lyases of *A. oryzae* MTCC 3782, *A. oryzae* MTCC 6993 with GenBank accession number KP86935, KP86938 and KP86939 respectively revealed prominent pectate lyase motifs.

The five conserved motifs of variable length namely 20, 21 and 50 revealed varied occurrence and motifs of 21 with sequence MLRERSNFPMPNLKITQTFAE was predominately present in 11 out of 14 sequences (Table 3). The pectate lyase domain was uniformly observed among all these motifs.

An insight into the sequences of PCR amplified pectate lyase genes representing different species and strains of *Aspergillus* using bioinformatics approach has been attempted in the present study.

In the present study, evidence for the presence of pectate lyase gene in *Aspergillus* species has been reported. Further attempt have to be made to look into the industrial applications of these enzymes. Pectinases have been extensively studied for their intervention in industrial applications. Bacterial pectate lyases have been exclusively reviewed recently [50-54]. Fungal pectate lyases are comparatively lesser explored though other pectinases namely pectin lyases and polygalacturonases from different fungal sources specially Aspergillus genera have been reported for retting and degumming of fibers [55].

The PCR amplificatied pectate lyases sequences of the present study show closeness with the pectinases of *A. niger, A. terreus, A. fumigates* [56] and *A. flavus* [57,58]. *A. niger* has been demonstrated to be promising source of alkaline thermostable pectinase. This property of enzymes can be exploted for several industrial applications like textile and food industry [59] Improved productions of pectinanses have also been attempted by several groups [60]. They have screened several fruit based substrates among which banana peel was found most efficient substrate for pectinase production. Though the current study has provided evidence for the existence of pectate lyases the protein needs to be purified and characterized for to study its usage in industrial applications.

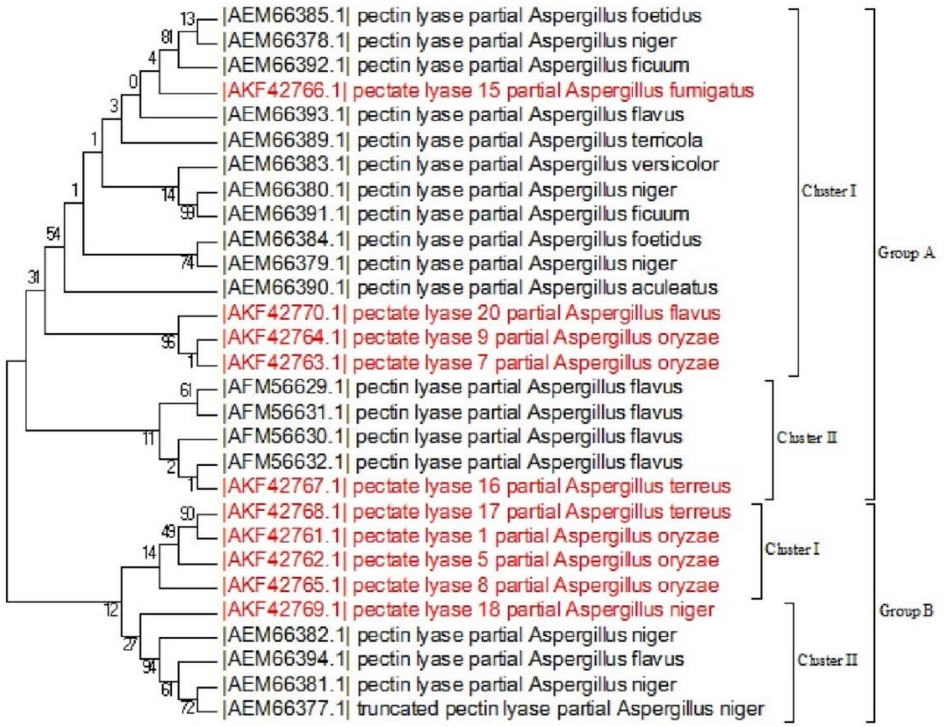

Fig. 4. Phylogenetic tree constructed using translated protein sequences of amplified pectate lyase genes representing different strains of *Aspergillus* (marked in red color) along with pectin lyases sequences using maximum likelihood method

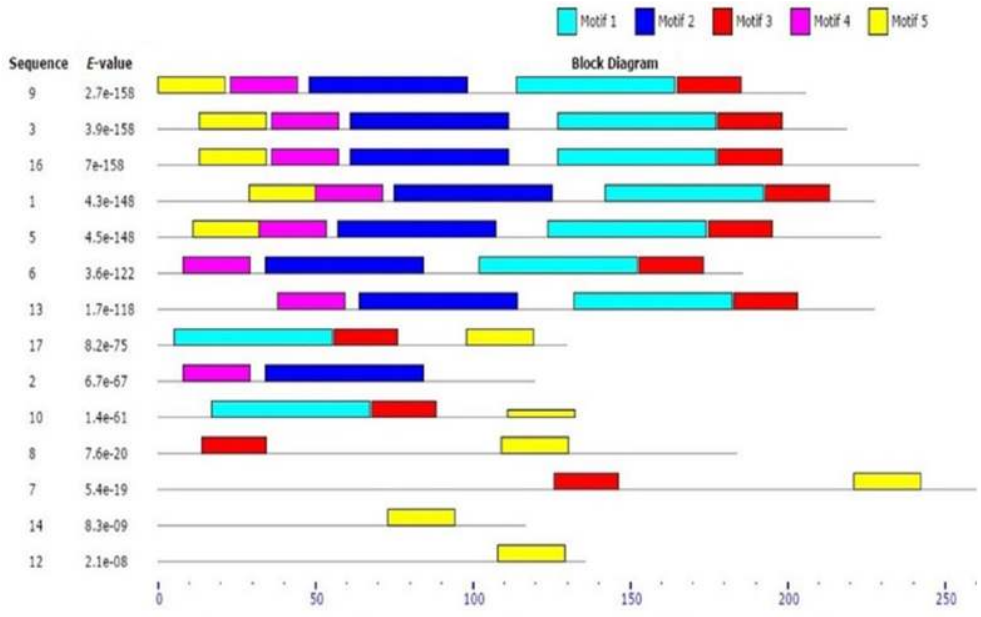

Fig. 5. Distribution of five conserved motifs among translated protein sequence of sequenced pectate lyase genes by MEME program

4. CONCLUSION

PCR amplification, cloning and expression of pectate lyases genes from bacterial sources have been extensively reported while only few of fungal pectate lyases genes are available in literature. An attempt has been made in the present study to standardize PCR amplification of pectate lyase genes using genomic DNA of different species and strains of *Aspergillus* as template. Further, the sequences of PCR amplified pectate lyases genes obtained were *in silico* characterized for homology search, multiple sequence alignment, phylogenetic tree and motif assessment. The existence of multigene families of pectate lyases needs to be extensively deciphered from sequenced fungal genomes to expand the potential applications. The pectate lyase genes characterized from different species and strains of *Aspergillus* in the present study should be cloned in appropriate vector, sequenced and optimized for expression in suitable host for elucidating diverse application studies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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