

## Research Article

# **In Silico Characterization of Pectate Lyase Protein Sequences from Different Source Organisms**

**Amit Kumar Dubey,<sup>1</sup> Sangeeta Yadav,<sup>1</sup> Manish Kumar,<sup>1</sup> Vinay Kumar Singh,<sup>2</sup> Bijaya Ketan Sarangi,<sup>3</sup> and Dinesh Yadav<sup>1</sup>**

<sup>1</sup>Department of Biotechnology, D.D.U Gorakhpur University, Gorakhpur 273 009, India

<sup>2</sup>School of Biotechnology, B.H.U, Varanasi 221 005, India

<sup>3</sup>Environmental Biotechnology Division, National Environmental Engineering Research Institute, Nehru Marg, Nagpur, Maharashtra 440 020, India

Correspondence should be addressed to Dinesh Yadav, dinesh\_yad@rediffmail.com

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A total of 121 protein sequences of pectate lyases were subjected to homology search, multiple sequence alignment, phylogenetic tree construction, and motif analysis. The phylogenetic tree constructed revealed different clusters based on different source organisms representing bacterial, fungal, plant, and nematode pectate lyases. The multiple accessions of bacterial, fungal, nematode, and plant pectate lyase protein sequences were placed closely revealing a sequence level similarity. The multiple sequence alignment of these pectate lyase protein sequences from different source organisms showed conserved regions at different stretches with maximum homology from amino acid residues 439–467, 715–816, and 829–910 which could be used for designing degenerate primers or probes specific for pectate lyases. The motif analysis revealed a conserved Pec\_Lyase\_C domain uniformly observed in all pectate lyases irrespective of variable sources suggesting its possible role in structural and enzymatic functions.

## **1. Introduction**

The enzymes hydrolyzing pectic substances ubiquitously present in the plant kingdom forming major components of middle lamella are referred as pectinases. The production, purification, biochemical characterization, and application of pectinases have been extensively reviewed [1–10]. The pectinases include polygalacturonases, pectic esterases, pectin lyases, and pectate lyases depending on their mode of action [1].

Pectate lyase (PL, EC 4.2.2.2) cleaves the  $\alpha$ -1,4 glycosidic bonds of polygalacturonic acid via a  $\beta$ -elimination reaction producing unsaturated  $\Delta$ 4, 5 bond at the nonreducing end of the polysaccharide and generates 4,5-unsaturated oligogalacturonates. Pectate lyase is widely distributed in diverse families of microorganisms and plants. The important members of bacterial family include *Erwinia carotovora*, *Bacillus polymyxa*, *Klebsiella*, *Yersinia*, *Cytophaga*, *Pseudomonas*, and *Xanthomonas* while in fungi *Aspergillus*,

*Fusarium*, and *Penicillium* are the most predominant source [9, 11–14].

A number of pectate lyase genes have been cloned, sequenced, and expressed from different source organism, namely, bacteria [15–22], fungi [23–25], yeast [26], nematode [27] and plants [14, 28].

The three-dimensional structures of various extracellular pectate lyase have been reported [29–36]. The pectate lyases, in general, have a parallel  $\beta$ -helix domain formed by parallel-strands folded into a large right-handed helix and a major loop region.

Amino acid sequence homology-based classification of pectate lyases into distinct families suggesting the possible evolution from different lineages has been reported [20, 35, 37–44]. In *in silico* analysis of pectin lyase protein sequences has been recently reported [45].

This paper reports *in silico* characterization of pectate lyase protein sequences from different source organisms for homology search, multiple sequence alignment, phylogenetic

TABLE 1: List of pectate lyase protein sequences with respective accession number from different source organisms.

Group	Total number	Accession number (Source organism name)
Nematode	06	AAQ09004.1[ <i>Meloidogyne incognita</i> ], AAL66022.1[AF455757 1[ <i>Meloidogyne javanica</i> ], AAF80747.1[ <i>Globodera rostochiensis</i> ], BAE48375.1[ <i>Bursaphelenchus mucronatus</i> ], BAE48371.1[ <i>Bursaphelenchus xylophilus</i> ], BAI44500.1[ <i>Aphelenchus avenae</i> ]
Plant	17	CAA47630.1[ <i>Nicotiana tabacum</i> ], NP 001150723.1[ <i>Zea mays</i> ], AAA33398.1[ <i>Lilium longiflorum</i> ], CAA70735.1[ <i>Zinnia elegans</i> ], AAQ84042.1[ <i>Malus x domestica</i> ], BAE48664.1[ <i>Prunus mume</i> ], AAY85180.1[ <i>Gossypium hirsutum</i> ], AAF63756.1[AF243475 1[ <i>Vitis vinifera</i> ], BAB59066.1[ <i>Salix gilgiana</i> ], gi 1256509 emb CAA63496.1[ <i>Musa acuminata</i> ], AAK66161.1[ <i>Fragaria x ananassa</i> ], BAF43573.1[ <i>Prunus persica</i> ], ACF40835.1[ <i>Manilkara zapota</i> ], ABG66729.2[ <i>Carica papaya</i> ], AAM63307.1[ <i>Arabidopsis thaliana</i> ], ABR26682.1[ <i>Fragaria chiloensis</i> ], ABD47739.1[ <i>Eucalyptus globulus</i> subsp. <i>Globules</i> ]
Fungi	10	AAA80568.1[ <i>Emericella nidulans</i> ], ABM60783.1[ <i>Penicillium citrinum</i> ], AAA75471.1[ <i>Glomerella cingulata</i> ], AAC64368.1[ <i>Fusarium oxysporum f. sp. lycopersici</i> ], ACS78057.1[ <i>Fusarium oxysporum f. cubense</i> ], EEY55044.1[ <i>Phytophthora infestans</i> T30-4], XP 749217.1[ <i>Aspergillus fumigatus</i> Af293], AAA80568.1[ <i>Emericella nidulans</i> ], ABM60783.1[ <i>Penicillium citrinum</i> ], EEY23761.1[ <i>Verticillium albo-atrum</i> VaMs.102]
Bacteria	87	AAB46398.1[ <i>Pseudomonas viridiflava</i> ], AAC60448.1[ <i>Pseudomonas marginalis</i> ], AAL56657.1[ <i>Pseudomonas syringae pv. glycinea</i> ], AAC41522.1[ <i>Xanthomonas campestris</i> ], AAM38405.1[ <i>Xanthomonas axonopodis pv citri</i> str 306], YP 001915548.1 [ <i>Xanthomonas oryzae PXO99A</i> ], AAY50632.1 [ <i>Xanthomonas campestris pv campestris</i> str 8004], YP 001314702.1 [ <i>Sinorhizobium medicae WSM419</i> ], ZP 06003184.1[ <i>Selenomonas noxia</i> ATCC 43541], ZP 04658159.1[ <i>Selenomonas flueggei</i> ATCC 43531], ACH58409.1[ <i>Bacillus subtilis</i> ], BAA05383.1[ <i>Bacillus sp. YA-14</i> ], ZP 05725973.1[ <i>Dickeya dadantii</i> Ech586], NP 866630.1[ <i>Rhodopirellula baltica</i> SH1], NP 228243.1[ <i>Thermotoga maritima</i> MSB8], ACR10769.1[ <i>Teredinibacter turnerae</i> T7901], ZP 04713986.1[ <i>Alteromonas macleodii</i> ATCC 27126], ZP 04334947.1[ <i>Nocardiopsis dassonvillei</i> subsp. <i>dassonvillei</i> DSM 43111], AAZ56201.1[ <i>Thermobifida fusca</i> YX], AAW84086.1[ <i>Bacillus halodurans</i> ], ACD11362.1[ <i>Bacillus pumilus</i> ], AAC38059.1[ <i>Pseudonocardia sp.</i> ], ZP 04608172.1[ <i>Micromonospora sp</i> ATCC 39149], ZP 05021985.1[ <i>Streptomyces svceus</i> ATCC 33406], YP 677777.1[ <i>Cytophaga hutchinsonii</i> ATCC 33406], CAA43401.1[ <i>Pectobacterium carotovorum</i> ], CAA47821.1[ <i>Erwinia chrysanthemi</i> ], ABF59812.1[ <i>Erwinia sp. BTC105</i> ], NP 866628.1[ <i>Rhodopirellula baltica</i> SH 1], ZP 01721007.1[ <i>Algoriphagus sp</i> PR1], ZP 01851785.1[ <i>Planctomyces maris</i> DSM 8797], ACL96719.1[ <i>Caulobacter crescentus</i> NA1000], AAO79220.1[ <i>Bacteroides thetaiotaomicron</i> VPI-5482], ABC76269.1[ <i>Syntrophus aciditrophicus</i> SB], YP 618077.1[ <i>Sphingopyxis alaskensis</i> RB2256], EER49733.1[ <i>Asticcacaulis excentricus</i> CB 48], YP 527779.1[ <i>Saccharophagus degradans</i> 2-40], YP 356933.1[ <i>Pelobacter carbinolicus</i> DSM 2380], CAB40884.1[ <i>Paenibacillus barcinonensis</i> BP23], YP 001615581.1[ <i>Sorangium cellulosum</i> 'So ce 56'], YP 002573715.1[ <i>Anaerocellum thermophilum</i> DSM 6725], ACU58213.1[ <i>Chitinophaga pinensis</i> DSM 2588], ZP 00943832.1[ <i>Ralstonia solanacearum</i> UW 551], CAG74004.1[ <i>Pectobacterium atrosepticum</i> SCRI1043], YP 432149.1[ <i>Hahella chejuensis</i> KCTC 2396], ZP 05805856.1[ <i>Streptomyces flavogriseus</i> ATCC 33331], ZP 05542020.1[ <i>Streptomyces griseoflavus</i> Tu4000], ZP 04688982.1[ <i>Streptomyces ghanaensis</i> ATCC 14672], ZP 05530610.1[ <i>Streptomyces viridochromogenes</i> DSM 40736], NP 827559.1[ <i>Streptomyces avermitilis</i> MA-4680], BAH85845.1[ <i>Streptomyces thermocarboxydus</i> ], ZP 05514668.1[ <i>Streptomyces hygrosopicus</i> ATCC 53653], YP 001506642.1[ <i>Frankia sp</i> EAN1pec], ZP 05011753.1[ <i>Streptomyces pristinaespiralis</i> ATCC 25486], ACV08000.1[ <i>Jonesia denitrificans</i> DSM 20603], ZP 04367351.1[ <i>Cellulomonas flavigena</i> DSM 20109], YP 001106556.1[ <i>Saccharopolyspora erythraea</i> NRRL 2338], ACU38032.1[ <i>Actinosynnema mirum</i> DSM 43827], AAL51034.1[AF454849 1[ <i>Klebsiella oxytoca</i> ], NP 149220.1[ <i>Clostridium acetobutylicum</i> ATCC 824], YP 003248772.1[ <i>Fibrobacter succinogenes ssp succinogens</i> ], ZP 04805707.1[ <i>Clostridium cellulovorans</i> .743B], YP002505581.1[ <i>Clostridium cellulolyticum</i> .H10], ZP 05495790.1[ <i>Clostridium papyrosolvans</i> .DSM.2782], AAG24437.1[AF303225 1[ <i>Bacillus alcalophilus</i> ], BAD63679.1[ <i>Bacillus clausii</i> .KSM-K16], YP 003091439.1[ <i>Pedobacter heparinus</i> .DSM.2366], EER72050.1[ <i>Sphingobacterium spiritivorum</i> .ATCC.33861], ZP 06173220.1[ <i>Brevundimonas subvibrioides</i> ATCC15264], ABJ82092.1[ <i>Candidatus solibacter usitatus</i> Ellin6076], EEX43407.1[ <i>Bacteroides finegoldii</i> DSM 17565], ZP 06083351.1[ <i>Bacteroides sp.</i> 2 1 22], YP 001196575.1[ <i>Flavobacterium johnsoniae</i> UW101], ACU08313.1[ <i>Flavobacteriaceae bacterium</i> 3519-10], YP 001817270.1[ <i>Opitutaceae bacterium</i> PB90-1], YP 003003775.1[ <i>Dickeya zeae</i> Ech1591], AAD25394.1[ <i>Azospirillum irakense</i> ], EER49094.1[ <i>Asticcacaulis excentricus</i> CB 48], YP 003061197.1[ <i>Hirschia baltica</i> ATCC 49814], AAG29353.1[AF279264 1[ <i>Cellvibrio japonicus</i> ], ZP 02927550.1[ <i>Verrucomicrobium spinosum</i> DSM 4136], ZP 03725417.1[ <i>Opitutaceae bacterium</i> TAV2], YP 003259879.1[ <i>Pectobacterium wasabiae</i> WPP163], ACT13209.1[ <i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i> PC1], AAM87379.1[AE013987 2[ <i>Yersinia pestis</i> KIM 10], YP 001175221.1[ <i>Enterobacter sp.</i> 638], ABR71284.1[ <i>Marinomonas sp.</i> MWYL1], YP 003243108.1[ <i>Geobacillus sp.</i> Y412MC10], YP 001018637.1[ <i>prochlorococcus marinus</i> str MIT 9303], YP 382660.1[ <i>Synechococcus sp.</i> CC9605]

TABLE 2: Distribution of motifs among 91 pectate lyase proteins sequences from different source organisms.

S. no.	Accession no.	Motif 1	Motif 2	Motif 3	Motif 4	Motif 5
1	AAB46398	+			+	
2	CAB40884					+
3	CAA47630	+	+	+	+	
4	CAA70735	+	+	+	+	
5	CAA43401				+	
6	CAA47821	+				
7	NP866630	+	+		+	
8	YP003003775				+	
9	ZP05725973	+			+	
10	NP866628	+	+		+	+
11	ZP05805856					+
12	YP002573715					+
13	YP001506642					+
14	NP001150723	+	+	+	+	
15	BAE48375					+
16	BAE48371					+
17	AAD25394	+				
18	ACV08000					+
19	ACU58213					++
20	AAC60448	+	+		+	
21	BAI44500					+
22	YP001314702	+			+	
23	AAQ09004					+
24	AAL66022.1 AF455757_1					+
25	AAF80747					+
26	AAC64368					+
27	AAA33398	+	+	+	+	
28	ACU38032					+
29	ACR10769	+	+	+		
30	ACU08313				+	
31	BAH85845					+
32	ACS78057					+
33	ZP04367351					+
34	AAW84086	+		+		
35	AAL56657	+	+	+	+	
36	AAK66161	+	+	+	+	
37	AAQ84042	+	+	+	+	
38	AAC41522	+			+	
39	AAA80568	+			+	+
40	ZP06003184	+			+	
41	ACH58409	+		+	+	
42	AAF63756.1 AF243475_1	+	+	+	+	
43	ZP04713986	+				
44	ZP04334947	+		+		
45	ABG66729	+	+	+	+	
46	ABR26682	+	+	+	+	
47	ACD11362	+		+		
48	XP749217	+			+	
49	BAA05383	+			+	
50	BAB59066	+	+	+	+	
51	ZP01851785	+	+			+

TABLE 2: Continued.

S. no.	Accession no.	Motif 1	Motif 2	Motif 3	Motif 4	Motif 5
52	ABM60783	+			+	+
53	BAF43573	+	+	+	+	
54	ABF59812	+				
55	AAY85180	+	+	+	+	
56	EEY55044					+
57	EEY23761				+	+
58	YP001615581				+	+
59	ZP05542020					+
60	ZP05530610					+
61	ZP05514668					+
62	ZP05011753					+
63	ZP04688982					+
64	YP001106556					+
65	NP827559					+
66	YP432149					+
67	ABD47739	+		+	+	
68	ZP00943832					+
69	AAM63307	+	+	+	+	
70	AAC38059	+	+		+	
71	AAA75471	+			+	
72	ACL96719	+	+			
73	AAZ56201	+		+		
74	AAY50632	+			+	+
75	AAM38405	+			+	
76	YP618077	+	+			
77	ZP04608172	+			+	
78	ZP05021985	+			+	
79	ZP04658159	+			+	
80	YP001915548	+			+	
81	NP228243	+	+		+	
82	YP356933		+			
83	YP677777	+			+	
84	ACF40835	+	+	+	+	
85	CAA63496	+	+	+	+	
86	AAO79220	+	+	+		
87	ABC76269	+	+			
88	ZP01721007	+	+			
89	BAE48664	+	+	+	+	
90	EER49733	+	+			
91	AAL51034.1 AF454849_1				+	
92	YP527779.1		+			

TABLE 3: Different motifs commonly observed in pectate lyases protein sequences with best possible match amino acid sequences.

Motif number	Width	Sequence	Occurrence in pectate lyase protein sequences
1	29	IAFNHFGEGLVQRMPRCRHGYFHVNNNDY	47
2	50	NPRPGTLRHAVIQDEPLWIVFKRDMVIQLKQELIMNSFKTIDGRGVNVHI	16
3	50	CITIQFVTNIIHGIHHDCKPTGNAMVRSSPSHYGWRTMADGDGIFG	16
4	50	HNSLSNCHDGLIDAIHGSTAITISNNYMTTHDKVMLLGHSDSYTQDKNMQ	39
5	49	SSSQTMTVDGGGARYAHDKVFQHNPGPTFVIKFNQVQDFGKLYRSCGNC	27

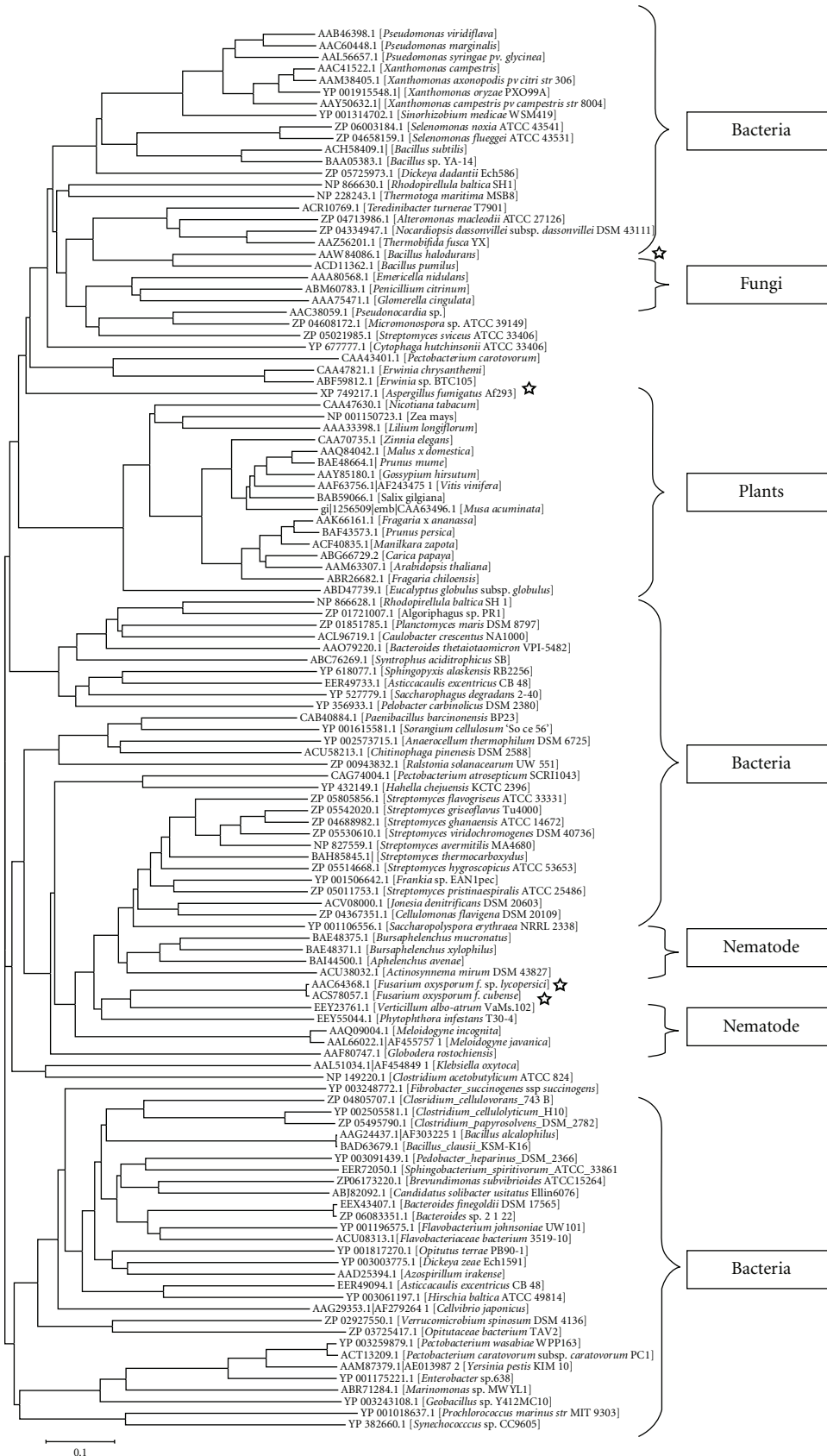


FIGURE 1: Phylogenetic tree of pectate lyase protein sequences from different source organism constructed by NJ method.



261821773	ref YP_003259879.7	CPFNAR	YTDKRI	NETSRR	GVQVGR	IS	-----A	DMGQPF	E-QQCP	FFATWG	SFLNSA	GNLLIF	SAS	-----	LDLYRM	KEDCAL	TPARHS			
251755133	ref ACJL71208.1	CPFNAR	YTDKRI	NETSRR	GVQVGR	IS	-----A	DMGQPF	E-QQCP	FFATWG	SFLNSA	GNLLIF	SAS	-----	LDLYRM	KEDCAL	TPARHS			
21969825	gb AA057379.1	CPFNAR	YTDKRI	NETSRR	GVQVGR	IS	-----A	DMGQPF	E-QQCP	FFATWG	SFLNSA	GNLLIF	SAS	-----	LDLYRM	KEDCAL	TPARHS			
146310147	ref YP_001175231.1	CPFNAR	YTDKRI	NETSRR	GVQVGR	IS	-----A	DMGQPF	E-QQCP	FFATWG	SFLNSA	GNLLIF	SAS	-----	LDLYRM	KEDCAL	TPARHS			
150837308	gb ABR71284.1	ALDNLN	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
220928672	ref YP_002505581.1	IDLHLL	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
256755010	ref EP_05495790.1	IDLHLL	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
2422260995	ref EP_04805707.1	INFLLN	GVQVA	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
10880924	gb AAG24437.1	IFDLYK	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
56909152	gb BAD63679.1	IFDLYK	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
25551067	ref YP_0039591439.1	WYLLAN	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
241862929	gb BEF7050.1	YELLKL	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
269924330	ref EP_06173320.1	LDLTLA	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
116232383	gb ABJ38092.1	FELLTK	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
260620536	gb EEK43407.1	FELLTK	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
262406802	ref EP_06093351.1	IQLTLK	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
146301984	ref YP_001196575.1	MLLELE	GVQVA	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
255342200	gb ACT098313.1	LCFLLE	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
251789054	ref YP_003003775.1	LCFLLE	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
4595937	gb AAR25394.1	YLLTLA	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
18241220	ref YP_001817270.1	LALLFA	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
241213081	gb BEF49094.1	LDLTLA	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
254295174	ref YP_003061197.1	LDLTLA	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
171912080	ref EP_02927550.1	LDLTLA	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
225159110	ref EP_03728417.1	LDLTLA	GVQVS	CCWDPV	VYDAGT	----	-----L	FWDRGQ	NKHLLE	LEENKC	SEFVNI	GNLVTE	SAG	-----	MLYVQL	HEKCAL	SWAFEL			
6223598	gb AAE46398.1	GPPYAK	S-----N	DVIWIR	LTVEHD	KD	-----E	EPIWD	ANRCSA	CWNSSE	YDCIIT	SG-ANN	YVQDHY	FTFTGCR	RIDDGK	CHANGC	VKKHSD	GALDVK	NGAMFY	
4095936	gb AAC60448.1	GPPYAK	S-----N	DVIWIR	LTVEHD	KD	-----E	EPIWD	ANRCSA	CWNSSE	YDCIIT	SG-ANN	YVQDHY	FTFTGCR	RIDDGK	CHANGC	VKKHSD	GALDVK	NGAMFY	
18032244	gb AALE5657.1	GPPYAK	S-----N	DVIWIR	LTVEHD	KD	-----E	EPIWD	ANRCSA	CWNSSE	YDCIIT	SG-ANN	YVQDHY	FTFTGCR	RIDDGK	CHANGC	VKKHSD	GALDVK	NGAMFY	
1197029	gb AAC4152.1	GPPYAK	S-----N	DVIWIR	LTVEHD	KD	-----E	EPIWD	ANRCSA	CWNSSE	YDCIIT	SG-ANN	YVQDHY	FTFTGCR	RIDDGK	CHANGC	VKKHSD	GALDVK	NGAMFY	
21103995	gb AA058405.1	GPPYAK	S-----N	DVIWIR	LTVEHD	KD	-----E	EPIWD	ANRCSA	CWNSSE	YDCIIT	SG-ANN	YVQDHY	FTFTGCR	RIDDGK	CHANGC	VKKHSD	GALDVK	NGAMFY	
188578619	ref YP_001915548.1	GPPYAK	S-----N	DVIWIR	LTVEHD	KD	-----E	EPIWD	ANRCSA	CWNSSE	YDCIIT	SG-ANN	YVQDHY	FTFTGCR	RIDDGK	CHANGC	VKKHSD	GALDVK	NGAMFY	
66575222	gb AAYS0632.1	GPPYAK	S-----N	DVIWIR	LTVEHD	KD	-----E	EPIWD	ANRCSA	CWNSSE	YDCIIT	SG-ANN	YVQDHY	FTFTGCR	RIDDGK	CHANGC	VKKHSD	GALDVK	NGAMFY	
150378107	ref YP_001314702.1	AYVVYN	S-----N	DVIWIR	LTVEHD	KD	-----E	EPIWD	ANRCSA	CWNSSE	YDCIIT	SG-ANN	YVQDHY	FTFTGCR	RIDDGK	CHANGC	VKKHSD	GALDVK	NGAMFY	
261876579	ref EP_06003184.1	NDVLVN	A-----D	NIIRIN	IMFSDP	YD	-----F	FDEWD	PNDCGE	CWNSMS	YDSITL	SG-CTH	IVWDHC	HFEDCG	ED	-----T	EYVPR	EYEHSD	GGLDIT	NGQIDL
238926399	ref EP_046581859.1	NDVLVD	A-----D	NIIRIN	IMFSDP	YD	-----F	FDEWD	PNDCGE	CWNSMS	YDSITL	SG-CTH	IVWDHC	HFEDCG	ED	-----T	EYVPR	EYEHSD	GGLDIT	NGQIDL
197293755	gb ACHE8409.1	GSPNPK	N-----D	NIWVIR	ISFQQA	YD	-----F	FQWDG	PTDSCS	CWNSSE	YDNITI	NG-ATH	IVWDHC	FTFDCG	HDFSCG	PYYVGR	KYGHSD	GLTDIA	NGAMFY	
450461	gb BAA05383.1	GSPNPK	N-----D	NIWVIR	ISFQQA	YD	-----F	FQWDG	PTDSCS	CWNSSE	YDNITI	NG-ATH	IVWDHC	FTFDCG	HDFSCG	PYYVGR	KYGHSD	GLTDIA	NGAMFY	
7593316	gb BAF43879.1	GSPNPK	N-----D	NIWVIR	ISFQQA	YD	-----F	FQWDG	PTDSCS	CWNSSE	YDNITI	NG-ATH	IVWDHC	FTFDCG	HDFSCG	PYYVGR	KYGHSD	GLTDIA	NGAMFY	
229298504	ref EP_04334947.1	LCIHRH	SS-----S	NIILQW	WYVWNV	KKS	-----G	CGSR	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
71916299	gb AAZ56501.1	LCIHRH	SS-----S	NIILQW	WYVWNV	KKS	-----G	CGSR	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
23893462	ref EP_04713986.1	LCIHRH	SS-----S	NIILQW	WYVWNV	KKS	-----G	CGSR	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
237683505	gb ACR10769.1	LCIHRH	SS-----S	NIILQW	WYVWNV	KKS	-----G	CGSR	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
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121584258	gb AEM60783.1	FCVLYK	SS-----S	NIWVIR	MSKCVL	YD	-----F	ADG	ICDRIG	QAS-SR	IVWDHC	ELSDSR	NS	-----	-----	-----	-----	-----	-----	-----
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238063463	ref EP_04608172.1	GCNLIS	NA-----S	WYVWNV	LTREVE	HTG	-----D	DAIS	ICSPSR	IVWDHN	ELVALS	NS	-----	-----	-----	-----	-----	-----	-----	-----
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98978749	gb ABF59812.1	FCWVIR	SS-----S	DVWVQN	MKSYGL	DGQ	-----A	KFD	QDWVIR	GD-SR	IVWDHC	ELPAAH	HCCD	-----	-----	-----	-----	-----	-----	-----
42336	emb CAA43401.1	ICPQVY	IG-----A	LVTGCR	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
226503329	ref NP_001150723.1	GMLTLV	QP-----N	NIWVIR	ISRNDI	VSSNED	-----G	GVTVIR	SDDHFC	WTVVSD	GDCIIT	FGS-TH	IVWDLR	SLRNGC	-----	-----	-----	-----	-----	-----
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139381	emb CAA7932.1	GCPIITL	QP-----N	NIILHQ	LHIKDC	KDQ-C	-----N	NAMVRS	SPRHGC	WTVVSD	GDCIIT	FGS-TH	IVWDLR	SLRNGC	-----	-----	-----	-----	-----	-----
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14289169	gb BAB59066.1	GCPIITL	QP-----N	NIILHQ	LHIKDC	KDQ-C	-----N	NAMVRS	SPRHGC	WTVVSD	GDCIIT	FGS-TH	IVWDLR	SLRNGC	-----	-----	-----	-----	-----	-----
1256509	emb CAA63496.1	GCPIITL	QP-----N	NIILHQ	LHIKDC	KDQ-C	-----N	NAMVRS	SPRHGC	WTVVSD	GDCIIT	FGS-TH	IVWDLR	SLRNGC	-----	-----	-----	-----	-----	-----
2463098	emb CAA70735.1	GCPIITL	QP-----N	NIILHQ	LHIKDC	KDQ-C	-----N	NAMVRS	SPRHGC	WTVVSD	GDCIIT	FGS-TH	IVWDLR	SLRNGC	-----	-----	-----	-----	-----	-----
14531296	gb AAK51621.1	GCPIITL	QP-----N	NIILHQ	LHIKDC	KDQ-C	-----N	NAMVRS	SPRHGC	WTVVSD	GDCIIT	FGS-TH	IVWDLR	SLRNGC	-----	-----	-----	-----	-----	-----
1																				

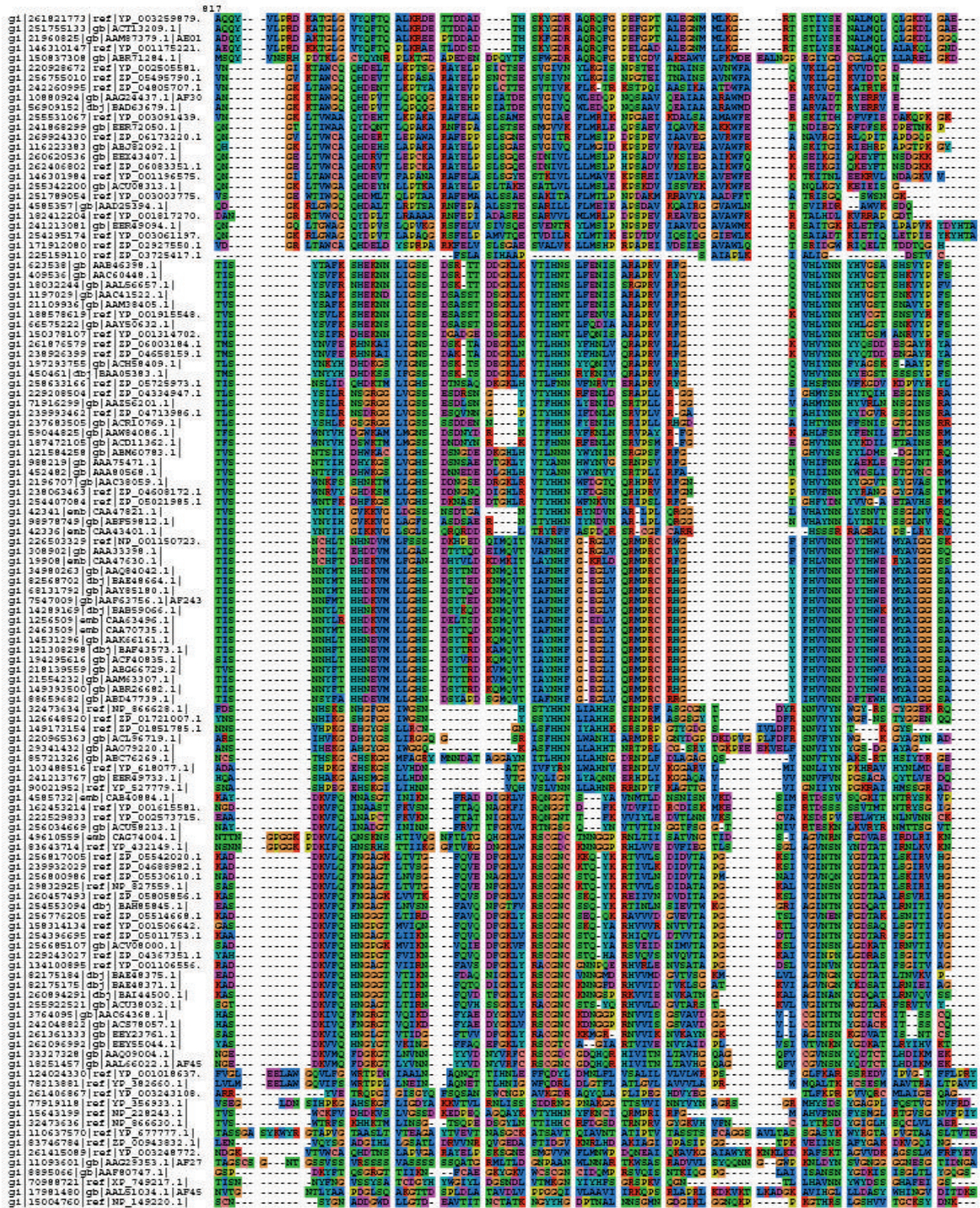


FIGURE 2: (a) Multiple sequence alignment of pectate lyase protein sequences showing maximum homology from amino acid residues 439–467. (b) Multiple sequence alignment of pectate lyase protein sequences showing maximum homology from amino acid residues 715–816. (c) Multiple sequence alignment of pectate lyase protein sequences showing maximum homology from amino acid residues 829–910.



tree construction, and motif analysis using various bioinformatics tools.

## 2. Materials and Methods

A total of 121 protein sequences of pectate lyases of different source organism available in GenBank were downloaded from NCBI (<http://www.ncbi.nlm.nih.gov/>). The accession numbers of pectate lyases protein sequences along with the source organism are listed in Table 1.

The program ClustalW [46] was used for multiple sequence alignment. Mega 4 was used for dendrogram construction by Neighbor-Joining (NJ) method [47]. For domain search, the Pfam site (<http://www.sanger.ac.uk/software/pfam/search.html>) was used. Domain analysis was done using MEME (<http://meme.sdsc.edu/meme/meme.html>) [48]. The conserved protein motifs deduced by MEME were characterized for biological function analysis using protein BLAST, and domains were studied with Interproscan providing the best possible match based on highest similarity score.

## 3. Results and Discussion

A total of 121 pectate lyases sequences from different source organisms subjected to phylogenetic tree construction revealed major clusters of bacterial, fungal, plant, and nematode pectate lyases. The pectate lyase from bacterial source was the predominant comprising of 87 accession numbers. The different accession of bacterial pectate lyase formed three major clusters as shown in Figure 1. The plant, fungal, and nematode pectate lyases formed separate clusters signifying the sequence-based similarity with reference to different source organisms. The multiple accessions of bacterial, fungal, plant, and nematode pectate lyases were placed closely in the clusters signifying the greater degree of sequence level similarity. Similar phylogenetic tree revealing clustering of pectin lyases protein sequences based on different source organism has been reported [45].

The multiple sequence alignment of these protein sequences revealed conserved regions at different stretches, namely, from 439–467, 715–816, and 829–918 amino acid residues (Figures 2(a), 2(b), and 2(c)). This region could be used for designing degenerate primers or probes for PCR-based amplification or hybridization-based detection of pectate lyase sequences from different source organisms.

A total of five motifs labelled as 1, 2, 3, 4, and 5 were observed in only 91 sequences when subjected to MEME. The distribution of these motifs among 92 pectate lyase accession number is shown in Table 2.

The motifs with width and best possible match amino acid sequences are shown in Table 3. All these motifs showed similarity with pec\_lyase\_C domain which is quite prevalent in pectin lyase sequences as reported earlier [45]. As the mechanism of pectin lyase and pectate lyase is quite similar, it is expected to show similar motifs. The motif 1 of 29 amino acid residues with sequence IAFN-HFGEGLVQRMPRCRHGYFHVVNNDY and motif 4 of 50

amino acid residues with a sequence HNSLSNCHDGLID-AIHGSTAITISNNYMTTHHDKVMLLGHSDSYTQDKNMQ were observed in 47 and 39 pectate lyase protein sequences (Table 3) signifying their possible role in the structural and catalytic attributes of pectate lyases.

Further when the motif best possible match amino acid sequence was subjected to BLAST to reveal its identity, it was observed that the motifs 1, 2, 3, and 4 represents Pec\_Lyase\_C superfamily while motif 5 represents pectate lyase superfamily. The exact function of these motifs in influencing the catalytic activity of the pectate lyase needs to be investigated.

The *in silico* characterization of pectate lyases protein sequences from different source organisms has revealed sequence level similarity specific for different groups which could be utilized for designing strategy for cloning the putative genes based on PCR amplification using degenerate primers.

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