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Research Article

SIGNATURE PATTERN ANALYSIS OF VARIOUS PECTINASES USING IN SILICO TOOLS

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ABSTRACT

Protein sequence of pectin lyases as available in NCBI was subjected for multiple sequence alignment, conserved domain analysis, topology and phylogenetic tree construction. Acetyl xylan esterase showed the maximum similarity indexed (99%) isolated from *A. flavus* having length of protein 307 with XP_002378019 accession number in hydrolytic depolymerase where as Polyhydroxybutyrate depolymerase showed the maximum similarity indexed raised from *Burkholderia pseudomallei* having length of protien 364 with YP_001077537 accession number. In depolymerase trans-membrane protein from *Burkholderia pseudomallei* showed presence of three helics located on 47-66, 97-120 and 178-201. Other proteins showed zero and one number of helics. Hydrolytic depolymerase showed four conserved motifs as Ferttrrygkpewgldxtel, Smtlggtidrrnptxayn, Rvamtvgndisgigqteaxh And Gvghgvfngsrfrseivpri as per sequence alignment where as estrase also showed four conserved motifs Sgltceqnfixksg, Vdpxxsfgshmgghgal, Sfghsmgghgal And Kipvvfrqegydhsy as per sequence alignment but depolymerase was not showed any conserved motifs.

INTRODUCTION

Pectinase is a general term for pectolyase, pectozyme as well as poly-galacturonase which are referred to infuse as pectic enzymes. Pectinases are divided into three groups according to their cleavage site as hydrolases consisting of polygalacturonase (PG; EC 3.2.1.15): pectin esterase (PE; EC 3.1.1.11) and lyase/trans-eliminases comprising pectin lyase, (PNL; EC 4.2.2.10). One of the most studied and widely used commercial pectinases is polygalacturonase which is a jelly-like matrix which helps to cement plant cells together along with other cell wall components, such as embedded cellulose or fibrils. Moreover, production, biochemical characterization as well as applications of PNL also have been assessed broadly. PE also catalyzed the galacturonan backbone of pectic substances de-estrification for releasing acidic pectins and methanol from methyl ester group. And then arised pectine substance from de-estrification acts upn by polygalacturonase or pectate lyase. PNLs is a more powerful tool for directly degradation of pectin polymers by b-elimination mechanism which formed 4,5-unsaturated oligogalacturonides however other pectinase enzymes acts as sequentially mechanism for degradation of pectin molecules completely. PGs are presents among fungi or bacteria where as PEs are mainly founds in plant as well as micro-organism related to plant pathogenicity; however PLs mostly formed by bacteria. Pectinases have been used in several conventional industrial processes, such as textile, plant fiber processing, tea, coffee, oil extraction, treatment of industrial wastewater, containing pectinacious material, etc. They have also been reported to work on purification of viruses and in making of paper. They are yet to be commercialized. Considering the broad range of applicability and their industrial potential, present study depicted the study of various classes of pectinase using respective sequences present in databases and *In silico* analysis of pectinase sequences present in database along their phylogenetic analysis. Conserved domain analysis of pectinase sequences may provide a clue for enhance the activity of enzymes and provide a data-set to design primers for molecular biology.

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MATERIALS AND METHODS

Pectinases Sequences: Protein sequence of different types pectinase proteins like eliminative depolymerase, hydrolytic depolymerase, esterase were retrived from NCBI database from Entrez. All these sequences were collected in text file i.e. Fasta Format.

Sequence Alignment of Pectinase Enzymes: Sequences correspond to depolymerase, hydrolytic depolymerase, esterase were aligned with Multalign online analysis tool. The alignment were analysed for Conserved Domain stored in text word file for further analysis.

Conserved Domain Analysis: Conserved Domain Analysis was performed with NCBI-CDD Search program. The detailed information about the conserved domain was performed in tables done by applying NCBI BLAST.

Topology Analysis: Topology of sequences corresponding to various protein families was performed with pectinase enzyme such as to depolymerase, hydrolytic depolymerase, esterase.

Phylogenetic Analysis: The phylogenetic tree is prepared from MEGA BLAST program. The protein sequence is selected from different file, selected from protein sequences of different kinds of pectinase enzymes like to depolymerase, hydrolytic depolymerase, estrase. The protein sequence is selected from fasta file alignment through Clustal W. The alignment is done through maximum likelihood method.

RESULTS AND DISCUSSION

Sequence Alingment

Depolymerase: The corresponding sequence of ten proteins depolymerase was used to identify its datadbase homologs using p blast in NCBI along with their similarity index. Polyhydroxybutyrate depolymerase showed the maximum similarity indexed raised from *Burkholderia pseudomallei* having length of protein 364 with YP_001077537 accession number. Minimum similarity indexed (91%) was observed in polyhydroxybutyrate depolymerase from *Burkholderia thailandensis* having ZP_02370017 accession number and 379 protein length.

Hydrolytic Depolymerase: Sequence of ten proteins hydrolytic depolymerases was used to identify datadbase homologs from NCBI using p blas as like depolymerase. Acetyl xylan esterase showed the maximum similarity indexed (99%) isolated from *A. flavus* having length of protein 307 with XP_002378019 accession number. Minimum similarity indexed (64%) was observed in hypothetical protein from *Phaeosphaeria nodorum* having XP_001805720 accession number and 300 protein length. Observed list of hydrolytic depolymerases was not shown 100% similarity indexed and maximum number of protein was found in fungi.

Esterase: By using p blast in NCBI, s-formylglutathione hydrolase showed the maximum similarity indexed (97%) isolated from *N. leucognys* having length of protein 282 with XP_003270110 accession number. Minimum similarity indexed (88%) was observed in hypothetical protein from *M. musculus* having XP_001475366 accession number and 282 protein length. Observed list of hydrolytic depolymerases was also not shown 100% similarity indexed and length of protein was observed 282 in all organisms.

Topology Analysis of Pectinase: All protein sequence of depolymerase, hydrolytic depolymerase and esterase family was analysed for their localization using HMMTOP server.

Table 1 List of top of 7 blast homology of depolymerase based on similarity to reference sequence from common source organisms

S. no.	Name of the protein	Organism	Length of protein	Accession no	Similarity (%)
1.	PHB depolymerase	*Burkholderia pseudomallei*	382	ZP_02494742	381/382 (99%)
2.	PHB depolymerase	*Burkholderia pseudomallei*	382	ZP_04891485	380/382 (99%)
3.	PHB depolymerase	*Burkholderia pseudomallei*	370	ZP_02460598	368/370 (99%)
4.	Unnamed protein product	*Burkholderia pseudomallei*	367	YP_105113	367/367 (100%)
5.	Polyhydroxybutyrate depolymerase	*Burkholderia pseudomallei*	364	YP_001077537	364/364 (100%)
6.	Unnamed protein product	*Burkholderia pseudomallei*	348	YP_111788	348/348 (100%)
7.	Polyhydroxybutyrate depolymerase	*Burkholderia thailandensis*	379	ZP_02370017	347/382 (91%)

Table 2 List of top of 10 blast homology of hydrolytic depolymerase based on their similarity to reference sequence from common source organisms.

S. no.	Name of the protein	*Organism*	Length of protein	Accession no	Similarity (%)
1.	acetyl xylan esterase	*Aspergillus flavus*	307	XP_002378019	305/307 (99%)
2.	Hypothetical protein	*Aspergillus terreus*	311	XP_001218465	230/310 (74%)
3.	Hypothetical protein	*Aspergillus nidulans*	306	XP_663697	232/309 (75%)
4.	acetyl xylan esterase	*Aspergillus clavatus*	308	XP_001267861	210/279 (75%)
5.	acetyl xylan esterase	*Aspergillus niger*	303	XP_001395572	215/275 (78%)
6.	acetyl xylan esterase	*Neosartorya fischeri*	368	XP_001262186	217/307 (71%)
7.	acetyl xylan esterase	*Aspergillus fumigatus*	371	XP_747458	210/283 (74%)
8.	acetyl xylan esterase	*Talaromyces stipitatus*	297	XP_002340391	201/274 (73%)
9.	Carbohydrate esterase family1 protein	*Thielavia terrestris*	284	XP_003653591	185/272 (68%)
10.	Hypothetical protein	*Phaeosphaeria nodorum*	300	XP_001805720	179/280 (64%)

Table 3 List of top of 10 blast homology of estrerases based on similarity to reference sequence from common source organisms.

S. no.	Name of the protein	Organism	Length of protein	Accession no	Similarity (%)
1.	s-formylglutathione hydrolase	*Nomascus leucognys*	282	XP_003270110	273/282 (97%)
2.	s-formylglutathione hydrolase	*Equus caballus*	282	XP_001490246	270/282 (96%)
3.	s-formylglutathione hydrolase isoform 5	*Pan troglodytes*	282	XP_509772	271/282 (96%)
4.	s-formylglutathione hydrolase	*Callithrix jacchus*	282	XP_002742723	270/282 (96%)
5.	s-formylglutathione hydrolase	*Loxodonta africana*	282	XP_003412663	270/282 (96%)
6.	s-formylglutathione hydrolase	*Callithrix jacchus*	282	XP_002753465	266/282 (94%)
7.	s-formylglutathione hydrolase	*Cricetulus griseus*	282	XP_003509949	262/282 (93%)
8.	s-formylglutathione hydrolase	*Cavia porcellus*	282	XP_003477527	259/282 (92%)
9.	s-formylglutathione hydrolase	*Callithrix jacchus*	282	XP_002746981	256/282 (91%)
10.	s-formylglutathione hydrolase	*Mus musculus*	282	XP_001475366	248/282 (88%)

Table 4 revealed the presence of six trans-membrane protein in depolymerase whereas four soluble proteins present in different species. Trans-membrane protein from *Burkholderia pseudomallei* showed presence of three helics located on 47-66, 97-120 and 178-201. Other proteins showed zero and one number of helics. Table 5 showed that hydrolytic depolymerase family have all soluble proteins and have no helics where as Table 6 showed all protein soluble and have no helics in estrase.

Sequence Alingment: Conserved motifs were identified in the pectinase protein sequence by using multalin. The protein sequence of depolymerase showed in Figure 1 no conserved motifs was found as per sequence alignments. Figure 2 showed four conserved motifs were found in hydrolytic depolymerase Ferttrrygkpewgldxtel, Smtlggtidrrnptxayn, Rvamtvgndisgigqteaxh and Gvghgvfngsrfrseivpri as per sequence alignment. Figure 3 showed four conserved motifs were also found in estrase Sgltceqnfixksg,

Table 4 The topology predicted using HMMTOP server. As per the table the mostly depolymerase are membrane bound except four are secreted i.e. soluble in nature.

S.No.	Name of protein	Organism	localisation	No. of helics	Location of helics
1.	depolymerase	*Burkholderia pseudomallei*	soluble	0	Nil
2.	depolymerase	*Burkholderia pseudomallei*	transmembrane	3	47-66, 97-120, 178-201
3.	depolymerase	*Burkholderia pseudomallei*	soluble	0	Nil
4.	depolymerase	*Pseudomonas sp.*	transmembrane	1	144-160
5.	depolymerase	*Rhodospirillum rubrum*	transmembrane	1	39-57
6.	depolymerase	*Micromonas sp.*	transmembrane	1	32-49
7.	depolymerase	*Micromonas sp.*	transmembrane	1	33-50
8.	depolymerase	*Burkholderia pseudomallei*	soluble	0	Nil
9.	depolymerase	*Ralstonia eutropha*	transmembrane	0	Nil
10.	depolymerase	*PHAZ_PSEFL*	transmembrane	1	181-198

Table 5 Topology predicted using HMMTOP server. As per table all hydrolytic depolymerase are soluble in nature.

S.No.	Name of protein	Organism	localisation	No. of helics	Location of helics
1.	esterase D	*Homo sapiens*	Soluble	0	Nil
2.	s-formylglutathione hydrolase	*Nomascus leucogenys*	Soluble	0	Nil
3.	s-formylglutathione hydrolase	*Pan troglodytes*	Soluble	0	Nil
4.	s-formylglutathione hydrolase	*Ailuropoda melanoleuca*	Soluble	0	Nil
5.	s-formylglutathione hydrolase	*Macaca mulatta*	Soluble	0	Nil
6.	s-formylglutathione hydrolase	*Canis lupus familiaris*	Soluble	0	Nil
7.	s-formylglutathione hydrolase	*Loxodonta africana*	Soluble	0	Nil
8.	s-formylglutathione hydrolase	*Callithrix jacchus*	Soluble	0	Nil
9.	s-formylglutathione hydrolase	*Cavia porcellus*	Soluble	0	Nil
10.	s-formylglutathione hydrolase	*Callithrix jacchus*	Soluble	0	Nil

Table 6 Topology predicted using HMMTOP server. As per table all esterase are soluble in nature.

S.No.	Name of protein	Organism	localisation	No. of helics	Location of helics
1.	Polyhydroxy alkanoate depolymerase	*Rhodopseudomonas palustris*	Soluble	0	Nil
2.	unnamed protein product	*Bradyrhizobium sp.*	Soluble	0	Nil
3.	unnamed protein product	*Bradyrhizobium japonicum*	Soluble	0	Nil
4.	unnamed protein product	*Rhodopseudomonas palustris*	Soluble	0	Nil
5.	unnamed protein product	*Rhodopseudomonas palustris*	Soluble	0	Nil
6.	Polyhydroxy alkanoate depolymerase	*Bradyrhizobiaceae bacterium*	Soluble	0	Nil
7.	putative intracellular PHB depolymerase	*Bradyrhizobium sp.*	Soluble	0	Nil
8.	unnamed protein product	*Bradyrhizobium japonicum*	Soluble	0	Nil
9.	intracellular PHB depolymerase	*Bradyrhizobium sp.*	Soluble	0	Nil
10.	Polyhydroxy alkanoate depolymerase	*Rhodopseudomonas palustris*	Soluble	0	Nil

Table 4 In this sequence the conserved domain identified 1super family is esterase-lipase and one multi-domain LpqC

S.no.	Conserved Domain Description
1.	
2.	
3.	
4.	
5.	

Vdpxxsfgshmgghgal, Sfghsmgghgal and Kipvvfrqegydhsy as per sequence alignment.

Conserved Domain Analysis: All the protein sequence of different pectinase enzyme were analysed for conserved domain in NCBI CDD search. LpqC depolymerase COG3509, Poly (3-hydroxybutyrate) depolymerase (Secondary metabolites biosynthesis, transport, and catabolism).

Table 5 In this sequence the conserved domain identified 2 super family and 1 multidomain.

S.no.	Conserved Domain Description
1.	
2.	
3.	
4.	
5.	

Table 6 In this sequence the conserved domain identified super family is esterase-lipase and 1 multi-domain COGO627.

S.no.	Conserved Domain Description
1.	
2.	
3.	
4.	
5.	

COG3509 is classified as a model that may span more than one domain. COG3509 is not assigned to any domain superfamily. PHB hydrolytic de-polymerase C-terminus; This family represents the C-terminus of bacterial poly(3-hydroxybutyrate) (PHB) de-polymerase. This degrades PHB granules to oligomers and monomers of 3-hydroxy-butyric acid. The enzymes of glycosyl hydrolase family 31 (GH31) occur in prokaryotes, eukaryotes, and archaea with a wide range of hydrolytic activities, including alpha-glucosidase (glucoamylase and sucrase-isomaltase), alpha-xylosidase, 6-alpha-glucosyltransferase, 3-alpha-isomaltosyltransferase and alpha-1,4-glucan lyase. All GH31 enzymes cleave a terminal carbohydrate moiety from a substrate that varies considerably in size, depending on the enzyme, and may be either a starch or a glycoprotein. In most cases, the pyranose moiety recognized in subsite -1 of the substrate binding site is an alpha-D-glucose, though some GH31 family members show a preference for alpha-D-xylose. Several GH31 enzymes can accommodate both glucose and xylose and different levels of discrimination between the two have been observed. Most characterized GH31 enzymes are alpha-glucosidases. In mammals, GH31 members with alpha-glucosidase activity are implicated in at least three distinct biological processes. The lysosomal acid alpha-glucosidase (GAA) is essential for glycogen degradation and a deficiency or malfunction of this enzyme causes glycogen storage disease II, also known as pompe disease. DepA: COG4553, Poly-beta-hydroxy-alkanoate depolymerase (Lipid metabolism) COG4553 is classified as a model that may span more than one domain. Esterase lipase superfamily: Esterases and lipases (includes fungal lipases, cholinesterases, etc.) These enzymes act on carboxylic esters. The catalytic apparatus involves three residues (catalytic triad): a serine, a glutamate or aspartate and histidine.These catalytic residues may responsible for the nucleophilic attack on the carbonyl carbon atom of the ester bond. In contrast with other alpha/beta hydrolase fold family members, p-nitrobenzyl esterase and acetylcholine esterase have a Glu instead of Asp at the active site carboxylate.

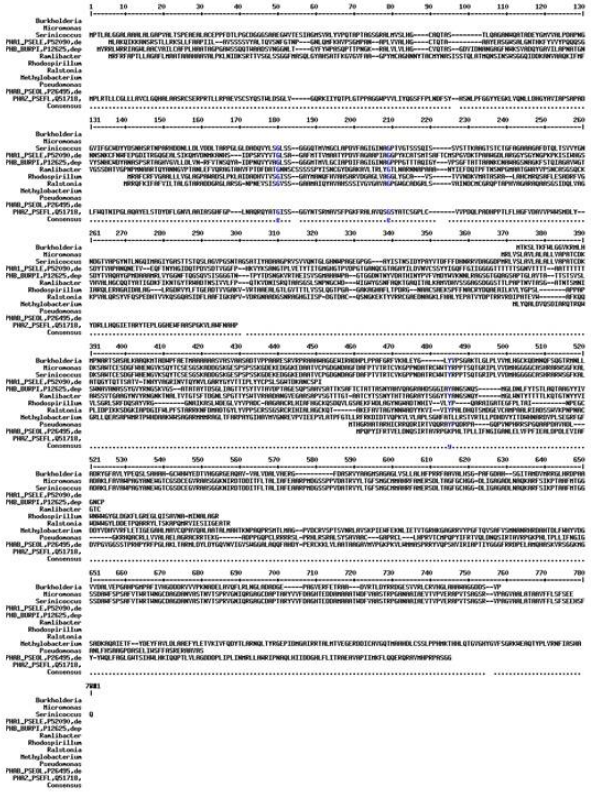

Figure 1 Sequence alignment of depolymerase. As per sequence alignment no conserved motifs are found.

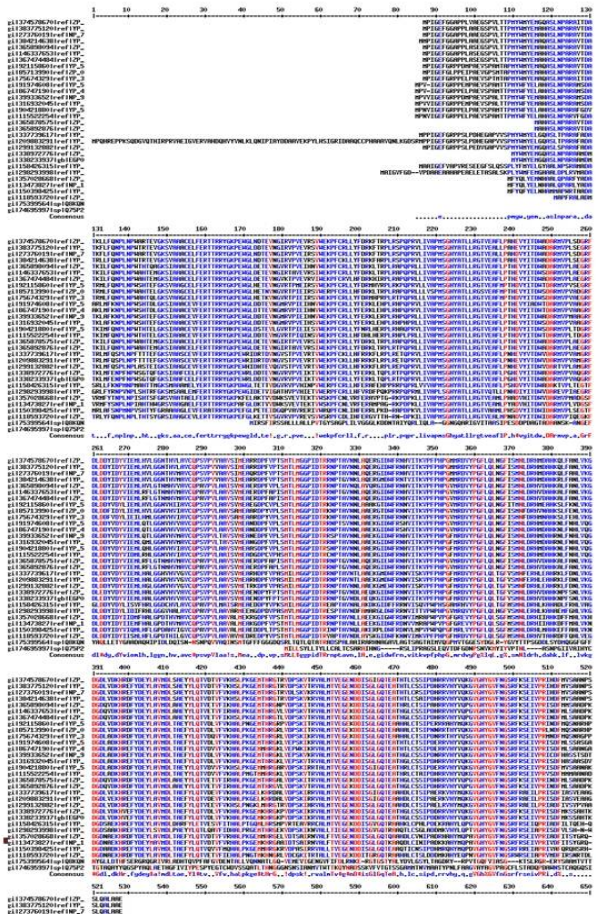

Figure 2 Sequence alignment of hydrolytic depolymerase

The lipase superfamily includes three vertebrate and three invertebrate (dipteran) proteins that show significant amino acid sequence similarity to one another.

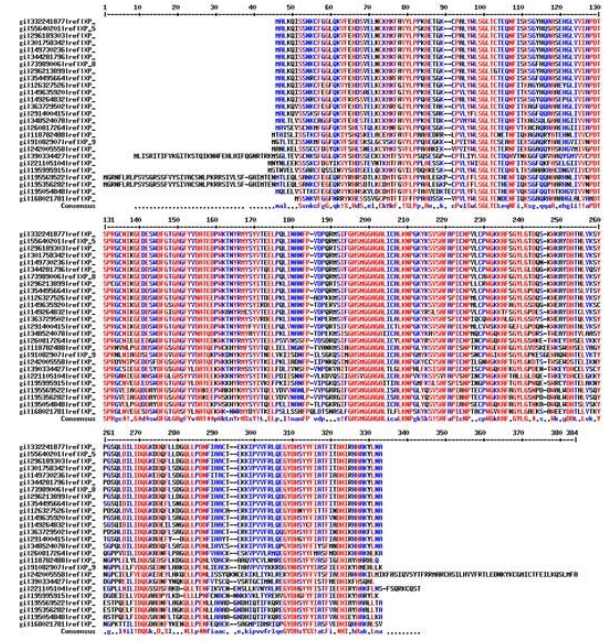

Figure 3 sequence alignment of esterase.

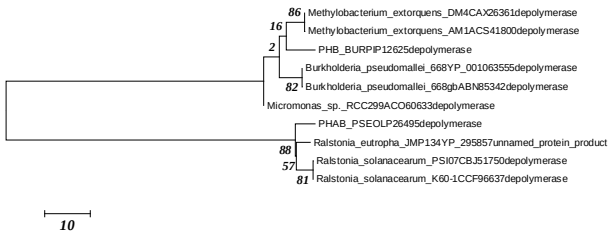

Figure 4 The phylogenetic tree of depolymerase.

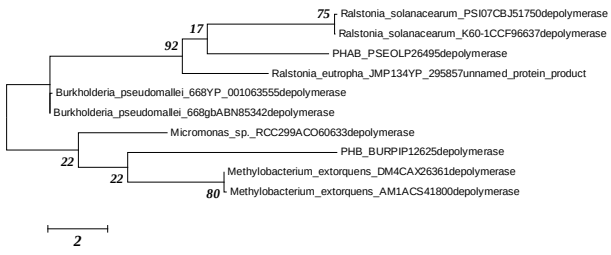

Figure 5 The phylogenetic tree of hydrolytic depolymerase.

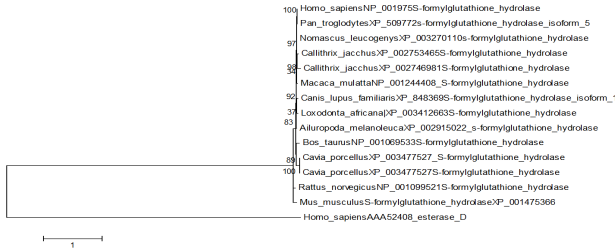

Figure 6 The phylogenetic tree of esterase.

The vertebrate proteins are lipoprotein lipase (LPL), hepatic lipase (HL), and pancreatic lipase (PL). The dipteran proteins are Drosophila yolk proteins 1, 2, and 3. We review the relationships among these proteins that have been established according to gene structural relatedness and introduce our findings on the phylogenetic relationships, distance relationships, and evolutionary history of the lipase gene superfamily. COG0627, Predicted esterase (General function prediction only) COG0627 is classified as a model that may span more than one domain. COG0627 is not assigned to any domain superfamily.

Phylogenetic Tree Analysis: Finally all sequence belongs to different families of pectinase were analysed for phylogenetic relationship by constructing phylogenetic tree in Megablast.

As per the tree of hydrolytic depolymerase have two different branches. It means two subfamilies are different where as the tree of esterase and depolymerase have one branche that means have one subfamily. From *In silico* analysis we have identified systematic pattern of each class of pectinase as well as we have observed all member of each class of pectinase possess almost similar character except few are dissimilar character. Even we have identified that even with in class; there are many sub groups which can be classified as new subclasses of cellulase after further analysis.

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