IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
Volume 9 | Issue 1 | January, 2014 | 148-153

RESEARCH ARTICLE

In silico analysis of pectinase enzymes from ascomycetes fungus

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SUMMARY

Pectinases are a big group of enzymes that break down pectic polysaccharides of plant tissues into simpler molecules like galacturonic acids. Pectinases are one of the most widely distributed enzymes in bacteria, fungi and plants. Some fungal species are used in industries as source of pectinases enzymes. It has long been used to increase yields and clarity of fruit juices. In the present investigation, total eleven protein sequences of pectinase enzyme from different fungal species of Ascomycetes were obtained from NCBI protein database and were considered for physico-chemical characterization including pI, EC, AI, GRAVY and instability index, motif discovery, motif family analysis and phylogenetic analysis. Three different motifs were discovered by MEME program where minimum motif width was 6 and maximum motif width was 50. All three discovered motifs were aligned using MAST tool which revealed the similarity between all of three submitted motif's sequence. The motif matches have shown a position p-value less than 0.0001. Each of the following 11 sequences has an E-value less than 100. There is only one major sequence clusters were constructed by phylogenetic analysis.

Key Words : *In silico*, Pectinase, Enzyme, Ascomycetes, Fungus

How to cite this article : Thakur, Anupsingh, Hande, Atul S. and Patil, Mahesh J. (2014). *In silico* analysis of pectinase enzymes from ascomycetes fungus. *Internat. J. Plant Sci.*, **9** (1): 148-153.

Article chronicle : Received : 07.10.2013; **Revised :** 26.10.2013; **Accepted :** 05.11.2013

The increasing energy demands have focused worldwide attention on the utilization of renewable resources, particularly agricultural and forest residues, the major components of which are cellulose, starch, lignin, xylan and pectin. These materials have attracted considerable attention as an alternative feedstock and energy source, since they are abundantly available. Several microbes are capable of using these substances as carbon and energy sources by producing a vast array of enzymes in different environmental niches (Antranikian, 1992; Kaur *et al.*, 2004). Pectinase production occupies about 10% of the overall manufacturing of enzyme preparations. Pectinolytic enzymes are widely used in the food industry for juice and wine production (Semenova *et al.*, 2006).

This review describes the pectinolytic enzymes and their substrates, the microbial pectinase production and characterization, and the industrial application of these enzymes. *In silico* approaches provide a viable solution to major drawbacks of experimental analysis of protein in regards to physicochemical and structural properties. Time requirement, high cost and the methods without amenable to high throughput techniques are the major drawbacks of experimental analysis of protein whereas Computationally based characterization of the features of the proteins found or predicted in completely sequenced proteomes is an important task in the search for knowledge of protein function.

MATERIAL AND METHODS

Eleven amino acid sequences of pectinase from different organisms of different fungal species of Ascomycetes were

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Table A : Protein sequences considered for the study

Organism	Enzymes	Accession number
Aspergillus kawachii	pectinase 2	gi|358373850|dbj|GAA90446.1|
	pectinase 3	gi|358372427|dbj|GAA89030.1|
	pectinase A	gi|358368561|dbj|GAA85178.1|
Aspergillus aculeatus	pectin methylesterase	gi|3220207|gb|AAC23565.1|
Aspergillus oryzae	polygalacturonase	gi|333943955|dbj|BAA03244.2|
Penicillium expansum	pectinase	gi|7388004|sp|O59925.1|PGLR_PENEN
Penicillium digitatum	pectinase	gi|7388011|sp|Q9Y718.1|PGLR_PENDI
Penicillium janthinellum	pectinase	gi|7388002|sp|O42824.1|PGLR_PENJA
Emericella nidulans	Endopolygalacturonase B	gi|294958172|sp|Q5B508.2|PGLRB_EMENI
	Polygalacturonase D	gi|74657032|sp|Q5AYH4.1
	Pectinase AN8327	gi|74656855|sp|Q5ATQ3.1|PGLR_EMENI

obtained from NCBI protein database and only 347 characters of each sequence were considered for motif discovery, motif family analysis and phylogenetic analysis (Bairoch and Apweiler, 2000) as shown in Table 1.

For physico-chemical characterization, theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction co-efficient (Gill and Von Hippel, 1989), instability index (Guruprasad *et al.*, 1990), aliphatic index (Ikai, 1980) and grand average hydropathy (GRAVY) (Kyte and Doolottle, 1982) were computed using the Expasy's ProtParam server (Gasteiger, 2005) (*http://us.expasy.org/tools/protparam.html*).

Motifs were identified in sequences using MEME program (Bailey and Elkan, 1995). All three discovered motifs were aligned using MAST program (Bailey and Gribskov, 1998) to judge the similarity between three motifs discovered. The multiple sequence alignment was performed using MUSCLE programme and CLUSTAL-W (Thompson *et al.*, 1994) program before we construct phylogenetic tree. The phylogenetic analysis was performed by UPGMA method using MEGA4 programme (Kumar *et al.*, 2008).

RESULTS AND DISCUSSION

The accession number of retrieved sequences along with the organism is listed in Table 1. The physio-chemical characterization were computed by Expasy's ProtParam tool as shown in Table 2. Isoelectric point (pI) value was calculated because that value of protein is stable and compact. pI value is the pH at which protein has no net charge. The computed pI

Table 1 : Physio-chemical parameters of pectinases enzymes

Organism	Emzymes	Accession number	Number of AA	M.wt	pI	-R	+R	EC	II	AI	GRAVY
Aspergillus kawachii	pectinase 2	>gi|358373850|dbj|GAA90446.1	362	37603.8	5.04	38	27	50920	31.58	78.12	-0.117
	pectinase 3	>gi|358372427|dbj|GAA89030.1	384	404260	4.03	58	18	47495	31.12	77.4	-0.147
	pectinase A	gi|358368561|dbj|GAA85178.1	370	38718.9	3.99	59	20	42900	34.95	73.24	-0.312
		gi|1223912|gb|AAB42153.1|	331	35681	4.23	38	16	64415	21.92	74.35	-0.191
Aspergillus aculeatus	pectin methylesterase	gi|3220207|gb|AAC23565.1|	378	38528.3	4.83	32	20	41410	25.44	77.57	-0.06
Aspergillus oryzae	polygalacturonase	gi|333943955|dbj|BAA03244.2	363	37651.3	4.51	40	23	42900	32.31	75.7	-0.194
Penicillium expansum	pectinase	gi|7388004|sp|O59925.1|	378	38028	6.9	22	22	36940	26.72	81.48	0.038
Penicillium digitatum	pectinase	gi|7388011|sp|Q9Y718.1	367	37651.2	8.04	30	32	36940	26.98	78.64	-0.134
Penicillium janthinellum	pectinase	gi|7388002|sp|O42824.1|	371	38326.9	4.27	46	23	45420	30.34	76.95	-0.174
Emericella nidulans	Endopolygalacturonase B	gi|294958172|sp|Q5B508.2	364	37857.2	7.52	33	34	39920	23.67	72.88	-0.226
	Polygalacturonase D	gi|74657032|sp|Q5AYH4.1	514	52731.6	4.51	54	19	34350	40.82	71.13	-0.132
	Pectinase AN8327	gi|74656855|sp|Q5ATQ3.1	380	39359.7	3.89	56	16	49890	29.55	74.39	-0.182

value of Pectinase from *Penicillium expansum*, *Penicillium digitatum* and *Emericella nidulans* (Endopolygalacturonase B) were greater than 7 (pI<7) indicates that these proteins were considered as basic. The computed isoelctric point (pI) will be useful for developing buffer system for purification by isoelectric focusing method. EC value and II value are shown in Table 1. Higher EC revealed that presence of higher concentration of light absorbing amino acids *i.e.* Cys, Trp and Tyr. Protein with low Instability index (smaller than 40) are stable (Guruprasad *et al.*, 1990).

AI value of pectinases protein are shown in Table 1. The very high aliphatic index of all pectinase protein sequences indicates that these antioxidant proteins may be stable for a wide temperature range.

Motif discovery result revealed that three motifs were discovered (Fig. 1). Three conserved motifs were found as shown in Fig. 8. The motif matches shown have a position p-value less than 0.0001(Fig. 8). The multiple sequence

gi|3220207|gb|AAC23565.1|	212	1.23e-57	IGTSTYVTIS	GATVYNQDDCVAVNSGENIYFSGGYCSGGHGLSIGSVGGRSDNTVKNVTF	VDSTIINSDN
gi|358368561|dbj|GAA85178.1|	200	1.09e-56	ISESTYITIT	GATVKNQDDCVAINSGENIYFSGGTCSGGHGLSIGSVGGRDDNTVKNVTF	IDSTVSDSEN
gi|7388002|sp|O42824.1|PGLR_PENJA	203	7.38e-53	ISESTGVTIR	NAVVKNQDDCIAINSGQNIYFTGGTCSGGHGLSIGSVGGRDDNTVKNVTI	TDSTVTDSAN
gi|294958172|sp|Q5B508.2|PGLRB_EMENI	196	1.24e-52	IGSSTYITID	GATVYNQDDCIAINSGEHITFTNGYCSGGHGLSIGSVGGRSDNTVKSVTI	SNSKVVDSQN
gi|358372427|dbj|GAA89030.1|	215	5.04e-52	IGESTYITIT	GAEIYNQDDCVAINSGENIYFSASVCSGGHGLSIGSVGGRDDNTVKNVTF	YDVNVLKSQQ
gi|7388004|sp|O59925.1|PGLR_PENEN	211	1.95e-51	IGDSDSITIT	GATVYNQDDCLAINSGTNIVFSGGYCSGGHGLSIGSVGGRSNNVVETVHI	SSTQVVNSQN
gi|333943955|dbj|BAA03244.2|	195	1.03e-50	VGSSTYINID	GATVYNQDDCLAINSGSHITFTNGYCDGGHGLSIGSVGGRSDNTVEDVTI	SNSKVVNSQN
gi|74656855|sp|Q5ATQ3.1|PGLR_EMEN	208	1.59e-49	IGESSNVVIT	GAKVYNQDDCVAVNSGTSITFSGGTCSGGHGLSIGSVGGRDDNTVDTVTF	KDSTVSNSVN
gi|358373850|dbj|GAA90446.1|	194	2.38e-48	VGNSVGVNII	KPWVHNQDDCLAINSGENIWFTGGTCIGGHGLSIGSVGDRSNNVVKNVTI	EHSTVSNSEN

Fig. 1 : Site of Block one

Fig. 2 : Block one show the motif location in each pectinase sequences

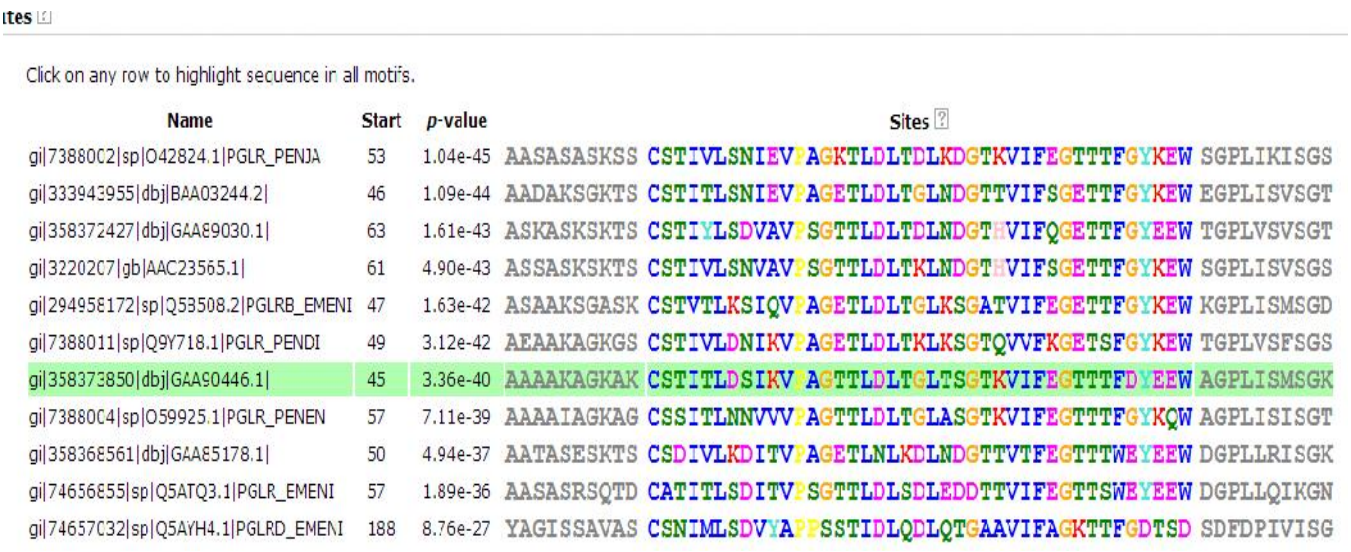

Fig. 3 : Site of block two

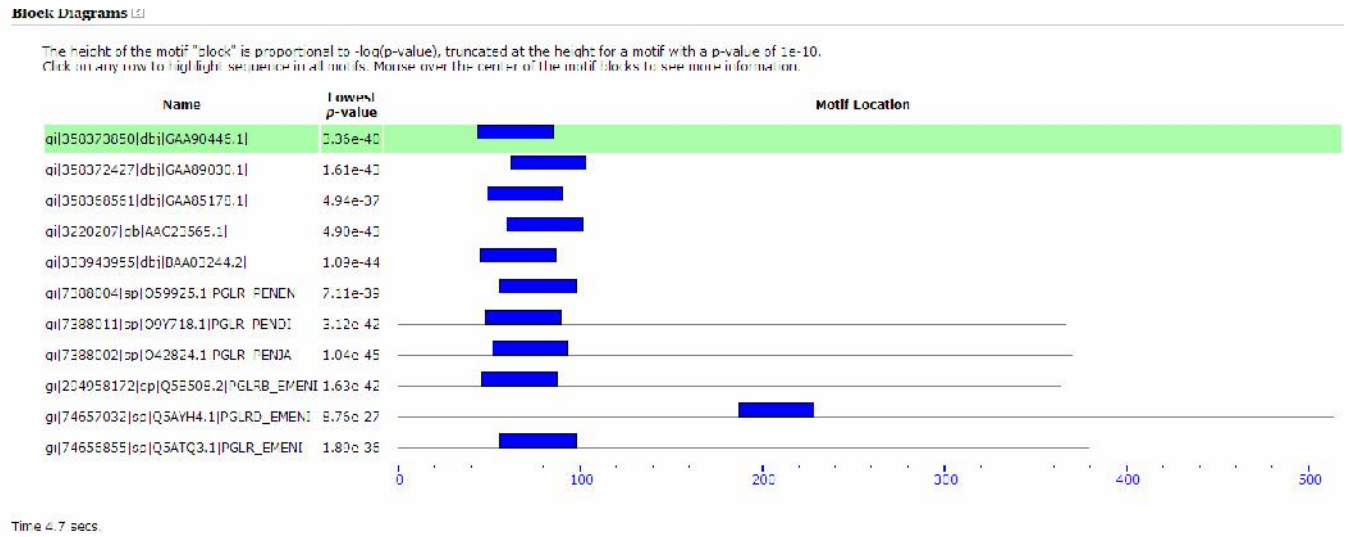

Fig. 4 : Block two show the motif location in each pectinase sequences

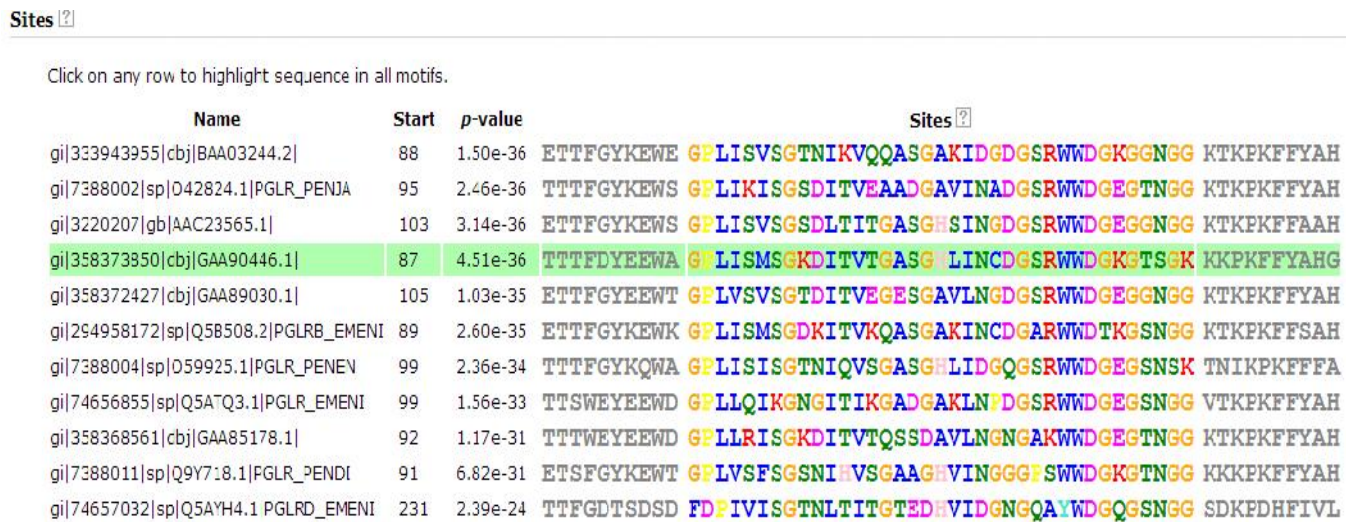

Fig. 5 : Site of Block three

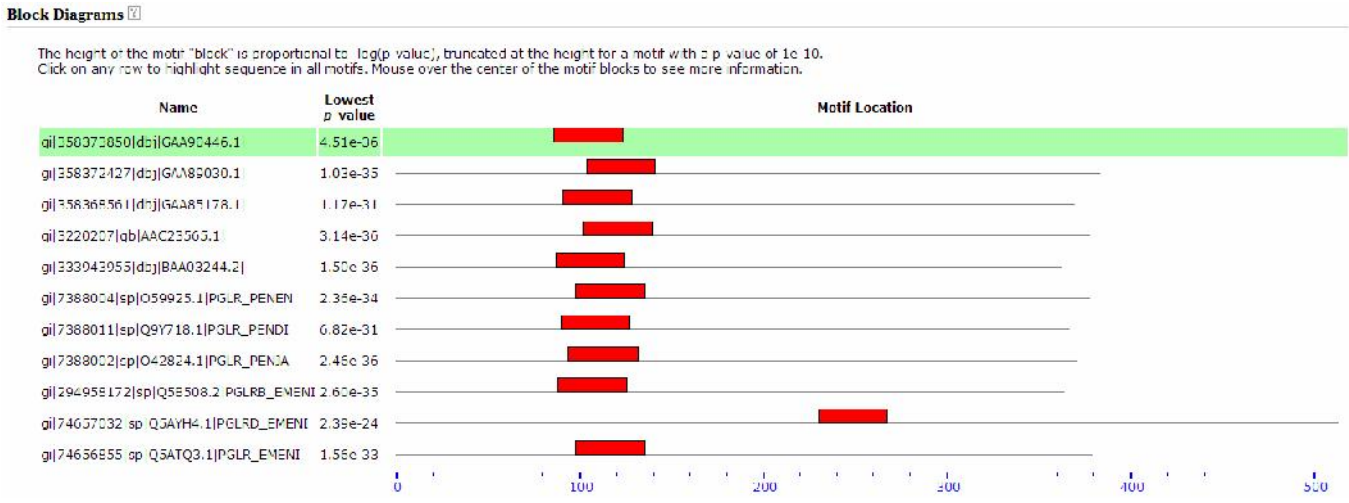

Fig. 6 : Block three show the motif location in each pectinase sequences

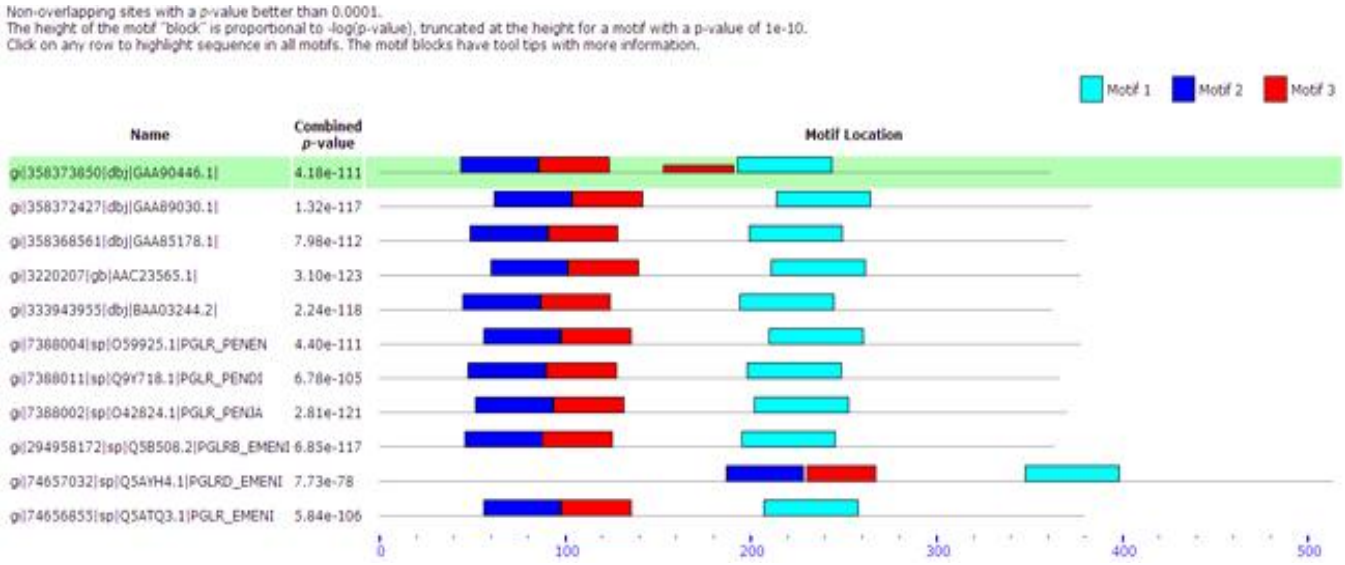

Fig. 7 : Combined block diagram show the motif location of each block

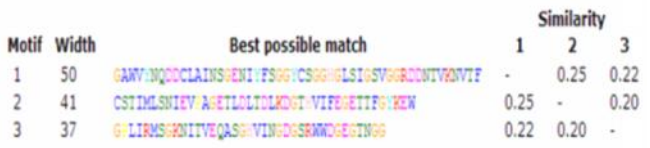

Fig. 8 : Best possible match diagram showing similarity between submitted motif

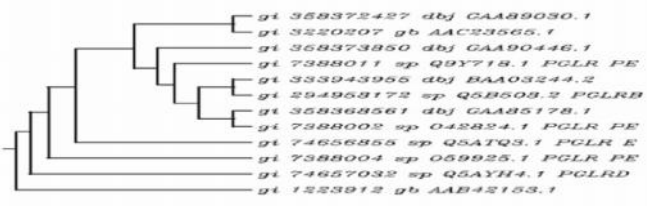

Fig. 9 : Phylogenetic analysis of all retrieved sequences of pectinase from different organisms of different fungal species of ascomycetes

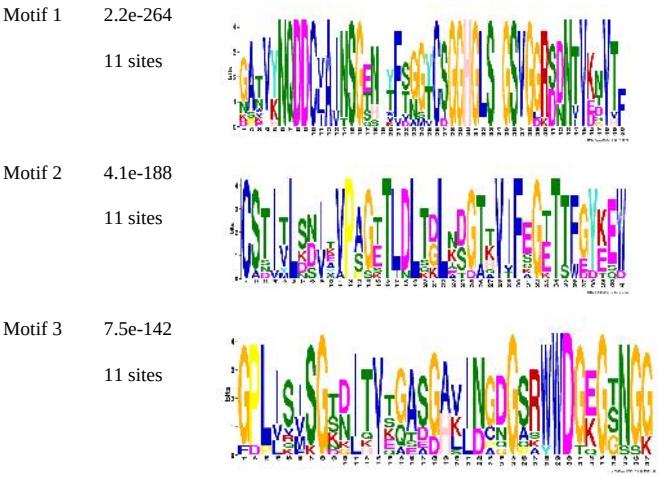

Fig. 10 : Conserved motifs of pectinases

alignment result showed some conserved regions in all aligned sequences.

Phylogeneic analysis for pectinases proteins from different Ascomycetes fungi was done by MEGA 4 tool. Results were shown in Fig. 9. There is single cluster to all proteins revealed that the functional motif are conserved in evolution.

Conclusion:

Motifs identification and similarity in a group of related sequences of pectinases showed the evolutionary relationships of function features among different organisms of fungal species of ascomycetes. This suggests that these motifs have an important function in the evolution of pectinases in different fungal species of ascomycetes.

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