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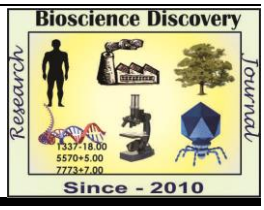
Research Article



An in-silico approach in motif and evolutionary analysis of lipases in insects

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Abstract

Lipases are esterase's that break down ester bonds of lipoproteins, fats and waxes present on insect surface. Lipases are serine hydrolases and so they do not require co factor. Phylogenetic analysis is the study of evolutionary relationship among molecules, phenotypes and organisms. In this study, we use Clustal-omega for multiple sequence alignment and created simple phylogeny by neighbor joining method. The phylogenetic tree of lipases from different insects demonstrated divergence patterns. Motifs were analysed by Motif finder and MEME analysis. A total of 26 conserved motifs were identified in lipase protein sequences by using MEME tool. Motif analysis of lipases showed anhydrolase, DUF676, DUF 2649 and staph haemo. DUF 676 belongs to putative serine esterase family whereas abhydrolase to alpha, beta hydrolase group. Phyre ^{2} was used for secondary structure prediction.

INTRODUCTION

Living organisms depend on proteins, lipids and carbohydrates for their physiological activities. But the proportion of requirement varies from species to species. Carbohydrases , lipases and proteases are the three main digestive enzymes involved in digestion process. Lipases (EC 3.1.1.3) seem to be very important not only for digestive role but also for esteratic activity and some experts consider lipases as class 3 of general esterase's (Estella *et al*., 2006). Lipases can be found in wide variety of plants, animals, insects and microorganisms. Plants lipases are mostly present in food reserve tissues (Vajanthi and Mumtaz 2002). In animals, the lipases are found in pancreas, on the surface of mucous cells of gastric mucosa and in insects, these enzymes are found mostly in plasma, salivary glands, muscles and fat bodies(Sandy *et al*., 2015). Lipases are widely used in detergent industries, food industries, pulp and paper industries, hydrolysis of fats and oils, and production of cosmetics and pharmaceuticals (Shaukat Ali *et al*., 2014). Lipases have key roles in insect lipid acquisition, storage and mobilisation and are also fundamental to many physiological processes underpinning insect reproduction, development, defense from pathogens and oxidative stress, and pheromone signaling (Horne *et al*., 2009). Storage lipids and membrane lipids are two types of lipids in insects. Storage lipids are triglycerides that are converted to mono and di glycerides in the midgut and stored for further metabolic processes (Zibaee *et al*., 2008). Although lipids produced from carbohydrates, dietary lipids are the most important parts of ingested food (Terra and Ferreira 2005). Phospholipids and glycolipids are the membrane lipids digested by phospholipases (Terra and Ferreira 2005). Triacylglycerol lipases (EC3.1.1.3) can hydrolyse the fatty acid ester bonds in presence of organic solvents like propanol so they can be

widely used in industrial areas like dairy, food, detergent and biofuel (Grillo *et al.,* 2007). Till now lipases were isolated and extracted mainly from microorganisms, fish, fungi, milk and plants (Grillo *et al.,* 2007; Degerli and Akpinar, 2002). Lipase production can be optimized on supplementation of media with mustard oil as carbon source and ammonium dihydrogen phosohate as nitrogen source (Tembhurkar *et al*.,2012). Insects rely on lipid reserves like lipophorins to survive during physiological non feeding periods or during egg development, flight and starvation (Ponnuvel *et al.,* 2003). In insects most of the stored fatty acids are released as 1,2-diacylglycerols and mobilization of lipid reserves from insect fat body is under the control of adipokinetic hormone (Zibaee *et al*., 2008). Lipases isolated and purified from the midgut of *Antheraea mylitta* has a molecular weight of 30kDa and the activity is highest at pH 8(Lakshmi and Benarjee 2016). Lipase activity is highest in the midgut region of *Periplanata Americana* (Oluwakemi *et al.,* 2014).Studies on *Bombus* shows that lipase activity is highest at 50°C and activity increases in presence of p-nitrophenyl (Jana Brabcova etal., 2013). Lipase activity increases in presence of Calcium, magnesium, sodium and ammonium salts (Shaukhat Ali *et al.,* 2014).

Phylogenetic analysis helps to identify and understand structure, putative function and evolution of true ortholog (Santosh Kodgire *et al*., 2015). Present work focus on identification of motifs and conserved regions in lipase sequences of insects obtained from NCBI and finding the evolutionary relationship by in silico analysis.

MATERIALS AND METHODS

Database search

The lipase sequence [accession no: 82792184] was retrieved from NCBI database, and the similarities of the lipase sequences are identified by performing BLASTp program.

Protein sequences alignment

Protein sequences are aligned globally to construct a multiple sequence alignment (MSA). The Multi sequences alignment was carried out by Clustal-omega. For multiple sequence alignments, the Clustal-omega version 1.2.4 was used to align the protein sequences. Fifteen sequences are used for the present research.The domains were obtained from the multiple sequence alignment. Clustal omega calculates the best match for the input sequences based on the parameters entered and generates an easy way to interpret report.

Motif analysis

Motifs for the aligned protein sequences were identified by incorporating the sequences in motif search (http://www.genome.jp/tools/motif/]tool and MEME version (4.11.2) [http:// memesuite. org/tools/meme tool. Protein domain prediction including determination of lipase, anhydrolase, duf 676, duf 2649 were identified by this tool.

Phylogenetic analysis

Clustal omega Multiple sequence alignment report displays the optimal alignment score, the alignment between sequences in a form such that the identities, similarities and differences can be clearly seen and a guide tree of the evolutionary relationships of aligned sequences (Figure 1).

Secondary structure prediction

The prediction was done by Phyre [2].

RESULTS AND DISCUSSION

NCBI and BLAST (blastp) literature search resulted in lipase protein sequences from 15 organisms. Literature also explained an overview of all sequences retrieved, including species specification, common protein aliases, and accession numbers. Present study explained the evolutionary relationship of the lipase protein from various organisms. Fifteen sequences of lipase proteins from various organisms were used in this phylogenetic study. Figure (1) demonstrates multiple sequence alignment that was determined by the Clustal-omega tool**.**

Sequence alignment

Clustal- omega is a multiple sequence alignment program for proteins. It produces biologically meaningful multiple sequence alignments of divergent sequences. It calculates the best match for the selected sequences, lines them up so that the identities, similarities and differences can be seen and a guide tree of the evolutionary relationships of aligned sequences (Figure 1).

Motif search

This method helps to find all known motifs present in given lipase sequences (Figure 2). Five different motifs were identified namely lipase,abhydrolase, DUF 676, DUF 2649 and staph haemo. DUF 676 belongs to Putative serine esterase family whereas Abhydrolase to alpha, beta hydrolase group.

Fig1: Multiple sequence alignment by clustal omega

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rutulus         VPHLVDLEEPIDEDVLYGRNGANNQYWLFTRRNPNNAQILRNGNAGSVTNSNYDRNRPLK
Papilio         VPQLVDLEEPVDEDVLYGRNGANNQYWLFTRRNPNNHQILVNGNANSVTNSNYNRNLPLK
aegeus          VPHLVDLHEPVDQDVLNGRNGANNQYWLFTRRNPNNHQILVNGNANSVSNSNYDRNRPLK
xuthus          VPHLVDLEEPVDEDVLNGRNGANNQYWLFTRRNPNNAQILRNGNAGSVTSSNYDRNRPLK
memnon          VPHLVDLEEPVDEDVLNGRNGANNQYWLFTRRNPNNAQILRNGNAGSVTSSNYDRNRPLK
Anthereae       VPHLVDLEEPAEEDILMSRNGANNQYWLFTRRNQNNHQVITNGNVNSIRNSNYNGSLPLF
mandarina       VPHLVDLEEPAEEDILMSRNGANNQYWLFTRRNQNNHQVITNGNVNSIRNSNYNGNLPLF
Bombyx          VPHLVDLEEPAEEDILMSRNGANNQYWLFTRRNQNNHQVITNGNVNSIRNSNYNGNLPLF
Samia           VPHLVDLEEPAEEDILMSRNGANNQYWLFTRRNQNNHQVITNGNVNSIRNSNYNGNLPLF
assamese        VPHLVDLEEPVDEEILNSRNGANNQYWLFTRQNPTNAQIITNGNANTIWSSNYRANRPTK
yamamai         --------RGVDEEILNSRNGANNQYWLFTRQNPTNAQIITNGNANSISSSNYRANRPTK
pernyi          VPHLVDLQAPIDESVVTGRNGANNQYWLYTRQNPTNAQVLVNGNANSISNSNYNGNRPTK
Spodoptera      KPHLVDLHEPADEEAI-ARNGANNQYWLYTRQNPTNAQVLVNGNANSVANSNYRANRGLK
Helicoverpa     VPHLVDLHEPADEALLASRNGANNQYWLFTRQNPTNAQIIVNGNANSIWNSNYRANRGLK
gea             VPHLVDLHEPADEALLASRNGANNQYWLFTRQNPTNAQIIVNGNANSIWNSNYRANRGLK
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Fig 2 : Domain regions present in lipase sequence obtained by motif search tool

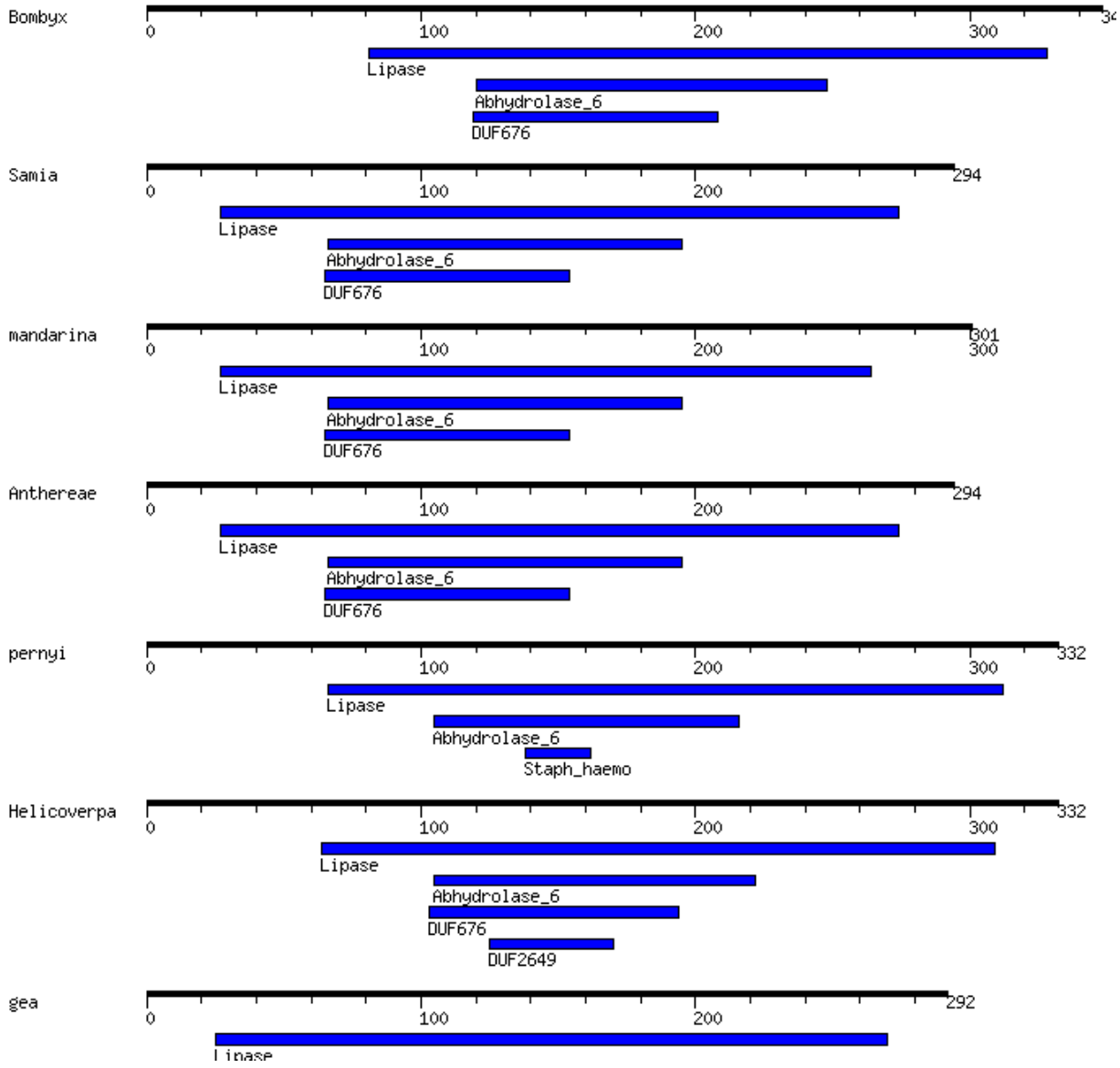


Fig 3: Conserved region of given sequence

Fig 4 : Motif location of lipase sequences in different organisms

Fig 5 : Phylogenetic analysis by neighbour hood method

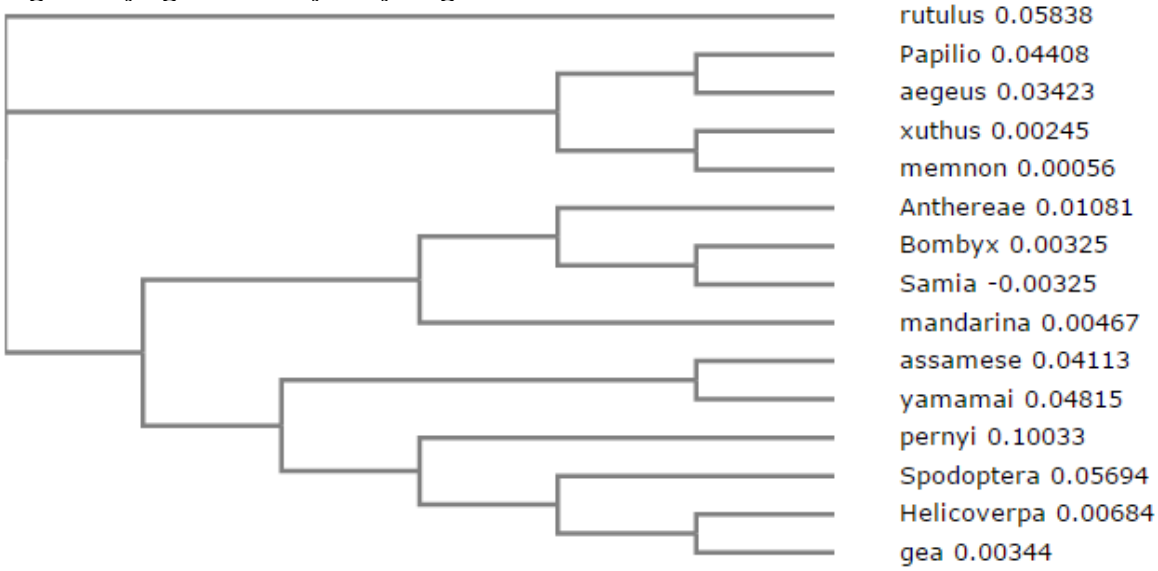
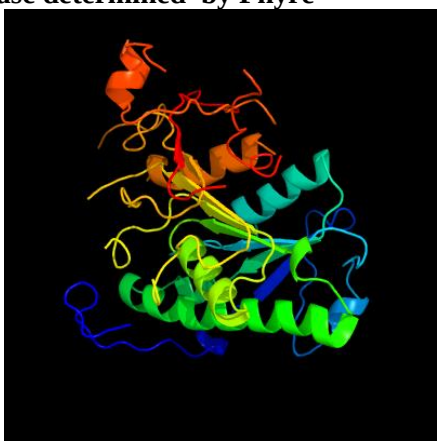


Fig 6 : Secondary sructure of Lipase determined by Phyre [2]



Motif Identification

This motif location was identified from the conserved region among the lipase sequences. The coloured portion represents the motif region. The height of the letter indicates its relative frequency at the given position in the motif (Fig 3).

Identification of important motifs from conserved region

Fig 4 shows that, a total of 26 conserved motifs were identified in lipase protein sequences by using MEME tool. The motifs have been represented in different colors. The lipase proteins are arranged according to their position in the phylogenic tree.

Construction of Phylogenetic tree of lipase

Phylogenetic studies shown the relatedness between selected organisms with their phylogenetic scores hase been shown in the figure 5. In bioinformatics, Neighbor Joining is a bottom-up clustering method for the creation of phenetic trees (phenograms) usually used for trees based on DNA or protein sequence data, the algorithm requires Neighbor joining takes as input a distance matrix specifying the distance between each pair of taxa. Present results shows that to establish the phylogenetic relationship between insects we have aligned 15 sequences with Clustal omega and constructed a phylogenetic tree. This is a neighbour joining tree, without distance corrections. Fig 5 shows that lipases are assigned to three different branches. The very long branch leading to rutulus. This long branch denotes high amount of species divergence. Eventhough ipases found in *papilio, aegeus, xuthus* and *memnon* are similar *papilio* and *aegeus* are on one taxon , *xuthus* and *memnon* are on one taxon. Lipases of *Anthereae, Bombyx, Samia* and *Mandarina* eventhough in same group *Bombyx* and *Samia* are on one taxon whereas *Anthereae* and *Mandarina* are diverged. Lipases of *assamese* and *yamamai* are on one taxon and helicoverpa and gea on same taxon but some divergence is noted in *pernyi* and spodoptera.

Secondary structure prediction shows that the confidence level was 100% with a coverage of 82%. Transmembrane helices was identified with 196 aa at N terminal and 211 aa at Cterminal positions (Fig 6). By using Phyre 2 a 3D structural model of lipase was built assuming that conserved parts of the amino acid sequences of different lipases represent conserved structural elements. Such conservation was described for residues involved in formation of the activesite, the disulphide bridges, salt bridges and some residues forming the protein core(Cygler *et al*., 1993). Present Phyre 2 structure shows that lipase contain an *α*/*β*-hydrolase fold and a catalytic triad comprising of residues Ser87, His286 and Asp264. Present results are in correlation with the studies on *Drosophila* in which when the active site is made accessible, lipase activity depends on a catalytic triad composed of the conserved consensus sequence (Gly-X1-Ser-X2-Gly) and two other residues (His and Asp)(Jacob *et al*., 2004). The enzyme shares several structural features with selected homologous lipases of all the selected organisms. Present findings are also similar to the studies performed on mammalian lipases where the active site "triad" residues are Ser159, Asp183, His 253 in mammalian lipases (Roger and Laura 2012). The present structure of lipase reveals a highly open conformation with a solvent-accessible active site. In silico analysis using the homology, phylogenetic analysis and 3-D structure prediction approach could help the computational biochemists to understand the fundamental information of lipase in insects.

In conclusion, the phylogenetic analysis is used to identify the evolutionary relationship among various organisms. This study is based on the overall in silico evaluation of lipase protein. We conclude that abhydrolase, DUF 676, DUF 2649 and staph haemo are the domains obtained from the motif results in insects.

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Web sites

http://www.ncbi.nlm.nih.gov.

http://www.genome.jp/tools/motif/

http:// memesuite.org/tools/meme tool

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