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Research article

MOLECULAR MODELING AND DOCKING STUDIES OF COLD ACTIVE LIPASE FROM *Pseudomonas fluorescens*

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ABSTRACT: Molecular Modeling is essential tool in the drug design process describes the generation, manipulation or representation of 3D structures of the molecules and associated physico-chemical properties while docking predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. A cold active lipase producing potential psychrophilic bacteria (GN) was isolated and identified by 16S rRNA molecular studies as *Pseudomonas vancouverensis*. Lipase gene from closely related species *P. fluorescens* was investigated for their functional role and *in silico* characterization using molecular modeling and docking studies. A 3D structure of lipase gene was generated with SWISS-MODEL and Discovery Studio 3.0. The stereochemistry of the constructed model of cold active lipase was subjected to energy minimization and the stereo-chemical quality of the predicted structure was assessed. The superimposition of the template (PDBID: 2Z8X) with predicted structure showed that weighted root mean square deviation of Cα trace between the template and the final refined model was 0.2 Å with a significant Z-score of 8.2 and sequence identity was 80.5%. Three ligands P-Nitrophenol, Acetate ion and Diethyl phosphonate were taken for docking calculation with generated structure. They were interacting on the functional motifs of predicted model. It has been observed that Leu26, Tyr29, Asn31, Asp33, Pro315 and Thr316 residues were involved in hydrogen bonding interactions with selected ligands. So these interacted residues can be used as prominent active binding sites and which was common to the predicted active site. Based on above investigations it has been found that *P. vancouverensis* lipase protein can play a similar role in lipid metabolic process and triglyceride lipase functional activity as reported for *P. fluorescens* lipase protein.

Keywords: SWISS-MODEL, Discovery Studio, Docking, Cold active lipase; Psychrophile, Pseudomonas; 16S rRNA.

INTRODUCTION

Lipase is most important enzyme belongs to the enzyme class of hydrolases (E.C.3.1.1.3) and hydrolyze triacylglycerols to fatty acids, diacylglycerol, monoacylglycerol and glycerol (Carriere *et al*., 1994). These enzymes break or modify the carboxyl ester bonds of lipids or its derivatives. The cold adapted lipases are largely distributed in microorganisms surviving at low temperature nearly 5ºC. Although a number of lipase producing sources are available, only a few bacteria and yeast are exploited for the production of cold adapted lipases (Joseph*,* 2006). Cold adapted lipases are mostly extracellular and are highly influenced by nutritional and physicochemical parameters such as temperature, agitation, pH, carbon source, nitrogen source, inducers, inorganic sources and dissolved oxygen. Submerged fermentation is the most common method used for cold adapted lipase production (Diekelmann *et al*., 1998; Lee *et al*., 2003).

The cold adapted lipases are probably structurally modified by an increasing flexibility of the polypeptide chain enabling an easier accommodation of substrates at low temperature. The fundamental issues concerning molecular basis of cold activity and the interplay between flexibility and catalytic efficiency are of importance in the study of structure-function relationships in the enzymes. Such issues are often approached through comparisons with the mesophilic or thermophilic counterparts, by site directed mutagenesis and 3D crystal structures (Narinx *et al*., 1997, Wintrode *et al*., 2000). A very low proportion of arginine residues as compared to lysines, a low content in proline residues, a small hydrophobic core, a very small number of salt bridges and of aromatic-aromatic interactions are the possible features of lipase for cold adaptation.

Similarly the weakening of hydrophobic clusters, the dramatic decrease of the proline content and of the ratio of Arg/Arg+Lys makes lipase active at low temperature (Gerday *et al*., 2000). It displays a small number of aromatic-aromatic interaction and salt bridges. The location of some salt bridges which are absent in cold lipase seems to be important for the adaptation to cold (Alquati *et al*., 2002). Few arginine residues were involved in the stabilizing intra-molecular salt bridges and a large proportion of them were exposed at the protein surface that may contribute to increased conformational flexibility of the cold active lipase. In addition to this the structural factors possibly involved in cold adaptation are the increased number and clustering of glycine residues and lower number of ion pairs and weakening of charge dipole interaction in α helices (Georlette *et al*., 2004; Gomes and Steiner, 2004). The catalytic activity of the cold active lipase is characterized by high plasticity. These structural adaptations may confer on the enzyme a more flexible structure, in accord with its low activation energy and its low thermo stability (Joseph *et al*., 2007).

Molecular modeling has become a valuable and essential tool to medicinal chemists in the drug design process describes the generation, manipulation or representation of three-dimensional structures of molecules and associated physico-chemical properties. The molecular modeling techniques are derived from the concepts of molecular orbitals of Hückel, Mullikan and 'classical mechanical programs' of Westheimer, Wiberg and Boyd.

Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex in the field of molecular modelling (Kumar *et al.,* 2013). The preferred orientation knowledge in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions. The associations between biologically relevant molecules such as proteins, nucleic acids, carbohydrates, and lipids play a central role in signal transduction. Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced (e.g., agonism vs antagonism). So this technique is useful for predicting both the strength and type of signal produced. It is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule (Kitchen *et al.,* 2004). Hence docking plays an important role in the rational drugs design. So the biological and pharmaceutical significance of molecular docking and its considerable efforts have been directed towards improving the methods used to predict docking.

METHODOLOGY

A potential psychrophilic bacteria *Pseudomonas* sp. was isolated from rice rhizosphere of Nainital Districts, Uttarakhand state, India. This strain was able to produce lipase at very low temperature.

16s rDNA identification of bacterial strain

16S rDNA gene sequence analysis and bacterial identification of selected bacterial strain was done from Microbial Type Culture Collection and Gene Bank (MTCC) at Institute of Microbial Technology, Chandigarh, India. The obtained nucleotide sequence compared with the public databases (NCBI) through BLAST analysis for their identification.

Retrieval of the target protein sequence

The protein sequence of extracellular lipase from most similar species was obtained from the protein sequence database of NCBI. It was ascertained that the three dimensional structure of cold active lipase from that species is not available in PDB (http://www.rcsb.org/) database, hence an attempt has been made in the present study to determine the structure using homology modeling.

Template identification

The NCBI BLAST was used to identify the template for modeling the three dimensional structure of cold active lipase from target protein sequence. The protein length and molecular weight were found out for obtained cold active lipase protein.

Model generation

The three dimensional structure of selected cold active lipase protein has been predicted using Discovery studio 3.0. A rough 3D model was obtained using generated alignment and modeling.

Refinement

The rough model generated was subjected to energy minimization using the steepest descent technique to eliminate bad contacts between protein atoms. Computations were carried out *in vacuo* with the GROMOS96 43B1 parameters set, implemented through Swiss-PdbViewer (http://expasy.org/spdbv/).

Evaluation

The backbone conformation of the rough model was inspected using the Phi/Psi Ramachandran plot obtained in the PROCHECK server (http://nihserver.mbi.ucla.edu/SAVES_3/saves.php). The rough model has been subjected to loop refining and energy minimization.

Superimposition of target and template

The structural superimposition of C^{α} trace of the template (2Z8X) and predicted structure of cold active lipase was performed using Combinatorial Extension of Polypeptides (http://cl.sdsc.edu/).

Active Site Prediction

After obtaining the final model, the possible binding sites of cold active lipase were searched using Q-SiteFinder (http://bmbpcu36.leeds.ac.uk/qsitefinder/). Ten binding sites were obtained for cold active lipase from Q-SiteFinder. These binding sites were compared to the active site of the template to determine the residues forming the binding pocket.

Docking studies

Based on previous studies in other micro-organisms, the following three compounds namely Acetate ion (CID: 175), Diethyl phosphonate (CID: 12977) and P-Nitrophenol (CID: 980) were found to effectively inhibitors for cold active lipase (Angkawidjaja *et al.*, 2010). To understand the interaction between cold active lipase and the three inhibitors, the protein-inhibitor complex was generated using Docking server (http://www.dockingserver.com/web).

RESULTS AND DISCUSSION

16s rDNA identification of bacterial strain

The lipase producing bacterial strain was identified as *Pseudomonas vancouverensis* through 16s rRNA gene sequence analysis (earlier reported by Gupta and Prakash, 2014). Sequenced amplicon has been submitted to NCBI database and accession no. JF508446 was obtained. Based on above information the cold active lipase protein sequences were retrieved from *Pseudomonas* sp. for homology modeling.

Retrieval of the target protein sequence and Template identification

The protein sequence of cold active lipase from *Pseudomonas fluorescens* was obtained from the protein sequence database of NCBI (Accession No: BAA36468). The NCBI BLAST was used to identify the template for modeling the three dimensional structure of cold active lipase from *Pseudomonas fluorescens*. Cold active lipase is 476 amino acids in length with a molecular weight of 50 kDa. The results yielded by NCBI BLAST against the PDB database revealed that cold active lipase from *Pseudomonas sp.* MIS38 (PDB ID: 2Z8X) with a resolution of 1.48 Å as a suitable template. The template and the target have 60% of residues identical with an *E*-value of 0.

Homology modeling of cold active lipase in *Pseudomonas fluorescens*

The absence of the three dimensional structure for *Pseudomonas fluorescens* cold active lipase in PDB prompted us to construct the 3D model shown in Fig. 1. The three dimensional structure provides valuable insight into molecular function and also enables the analyses of its interactions with suitable inhibitors. Among the three conformations generated, the one with the least modeler objective function value was considered to be thermodynamically stable and chosen for further refinement and validation.

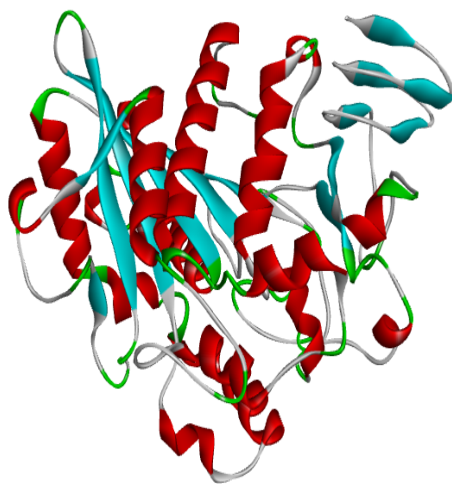

Fig. 1: 3D structure of cold active lipase obtained after energy minimization. The α-helix is represented by red, β-sheet by cyan and loops by grey lines.

Validation of the predicted structure

The stereochemistry of the constructed model of cold active lipase was subjected to energy minimization and the stereo-chemical quality of the predicted structure was assessed. Ramachandran plot of cold active lipase built and plot calculations on the 3D model of cold active lipase were computed. The Ramachandran plot for the model showed 90.6% of the residues in the core region, 8.8% residues in the allowed regions and 0.6%, i.e. one residue in the disallowed region (Fig. 2, table 1). The results of Ramachandran plot indicates that the model generated had two residues (SER207 and SER402) in the disallowed region, occurring in the loop of secondary structure. Hence, it was subjected to loop refinement and further energy minimization. In an analysis of the final model, 90.6% of the residues were found to occupy the core region. The residue in the disallowed region had been shifted to the generously allowed region, thereby optimizing and stabilizing the overall conformation of the predicted structure.

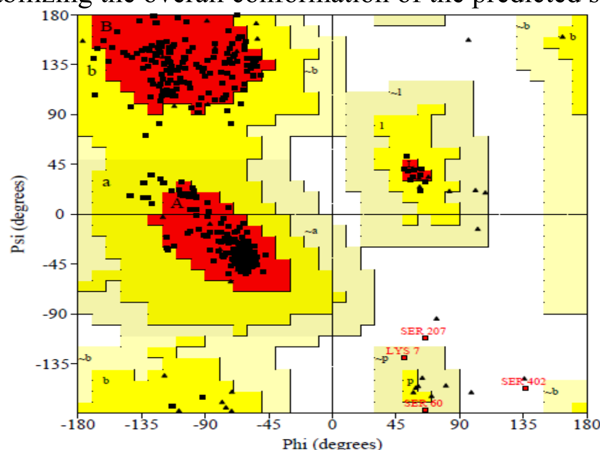

Fig 2: Ramachandran plot of cold active lipase built using PDBSum server.

Table 1: Ramachandran plot details after energy minimization.

Plot statistics	After energy minimization
% of residue in most favored regions	90.0
% of residue in the additionally allowed	8.8
% of residue in the generously regions	0.6
% of residue in dis-allowed regions	0.6
% of non-glycine and non-proline residues	100

Superimposition of the template with predicted structure

The predicted structure of cold active lipase was superimposed on template from *Pseudomonas* sp. MIS38 (PDB ID: 2Z8X). The alignment length was 410 with 0 gaps. The weighted root mean square deviation of Cα trace between the template and the final refined model was 0.2 Å with a significant Z-score of 8.2 and sequence identity was 80.4% shown in Fig. 3.

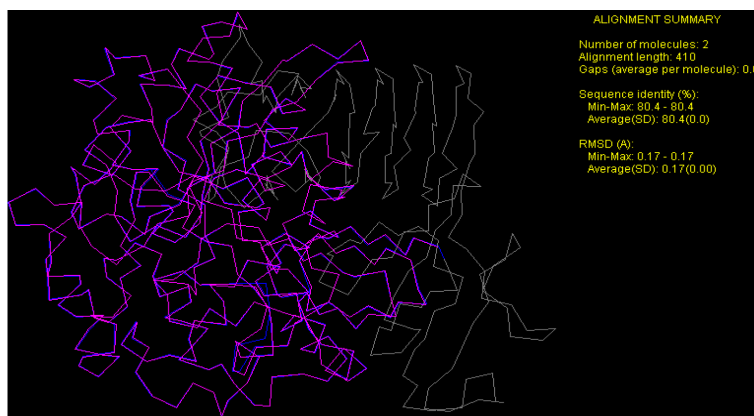

Fig. 3: Superimposition between Target and Template structure

Active site identification of cold active lipase

Among the ten binding sites obtained from Q-Site Finder, site 1 is highly conserved with the active site of the template. It is also found from the results of multiple sequence alignment (Fig. 4) and secondary structure prediction that the residues in site 1, Asp33, Phe36, Ala37, Val38 Tyr40, Gln41, Tyr252, Ser287, Phe288, Asn289, Asp290, Ile311, Ser312, Leu314, Pro315, Thr316, Ala317, Asp320, Gly321, Arg324, Ala343, Asn344, Leu345, Ser346, Asp347, Arg350, Val355, Ser374, Asp375, Asp378 are conserved with the active site of the template. Thus, site 1 has been chosen in this study as the most favorable site for docking and the other sites are not further discussed. *Pseudomonas fluorescens* binding pocket containing the residues Leu26, Tyr29, Asn31, Asp33, Asn34, Phe36, Ala37, Val38, Tyr40, Gln4, Pro315 and Thr316 were conserved and therefore considered for docking analysis. The residues forming the binding pocket are shown in Fig. 5.

```
ZP_07775481.1_Pseudomonas_fluo    LGVEGSKALF ADAMAITLYS YHNLDNGFAV GYQHNGLGLG LPATLVGALL
YP_002872716.1_Pseudomonas_flu    LGTEGSKALF ADAMAITLYT YHNLDNGFAV GYQHNGLGLG LPATLVGALL
BAA36468.1_Pseudomonas_fluores    LGTEGSKALF ADAMAITLYS YHNLDNGFAV GYQHNGFGLG LPATLVGALL
YP_004354131.1_Pseudomonas_bra    LGTTESKALV SDAMAIMLYS YHNLDNGFAA GYQHNGFGAG LPATLVTALL
YP_607820.1_Pseudomonas_entomo    LGSSASSALY KDAMALAMYA YHGIDDGFAA GYQQHGFGLG LPATLVKALI
ZP_06189896.1_Serratia_odorife    LDDAKSKTLF SDALAISTYA YHNIDNGFDE GYHHYGFGLG LPFTLVTALI
YP_001478438.1_Serratia_protea    LDEAKSKALF TDALAISTYA YHNIDNGFDE GYHNTGFGLG LPLTLVTALI
NP_929565.1_Photorhabdus_lumin    FDDTTSKELF SDAKTISGYA YHNLGSGFAE GYYNYGFGLG MPLTLLTAIL
YP_003041132.1_Photorhabdus_as    FDDATSKELF SDAKAISGYA YHNLGSGFAE GYYNYGFGLG MPLTLLKAII
YP_003612281.1_Enterobacter_cl    --GEKNTQLI KDALTIESIN FG------AA TYADYTY--- ----------
ZP_05083622.1_Pseudovibrio_sp.    YKGNDARQLM SDALDLMLYS YHGLDDALGS AYQANGFS-- -LATLVGTLW
YP_001280842.1_Psychrobacter_s    YSSEEAAKLV DDASRLSAYT NAVSFFGFIP GADALNVVG- ----------
ZP_08387820.1_Sphingomonas_sp.    YSVTESAELA NTTLQLATFG QLDRIFG-LP VAQLANTFGN ----------
ZP_07775481.1_Pseudomonas_fluo    NIVSFNDHYA STLWNVLPFS IVNLPT-WVS HLPTGYGDGM TRILDSGFYE
YP_002872716.1_Pseudomonas_flu    NIVSFNDHYA STLWNVLPFS IANLPT-WLS HLPTGYGDGM TRIVESGFYE
BAA36468.1_Pseudomonas_fluores    NIVSFNDHYA STLWNVLPFS IVNVPT-WIS HLPTAYGDGL TRVLDSQFYD
YP_004354131.1_Pseudomonas_bra    NIVSFNDHYA STAWNVLPFS ILNIPT-WIS HLPTGYGDGM GRIMGSVFYD
YP_607820.1_Pseudomonas_entomo    NIVNFNDHYA SDAWNLLPFS IANIAT-WLS HLPAAYTDGL GRVVDSSFYQ
ZP_06189896.1_Serratia_odorife    NIVNFNDHYA SAAWNILPFS ILNLPT-WLS HLPFFYQDGL MRVLNSTFYS
YP_001478438.1_Serratia_protea    NIVNFNDHYA SAAWNILPFS ILNIPT-WLS HLPFFYQDGL MRVLNSEFYS
NP_929565.1_Photorhabdus_lumin    NIVNFNDHYA SDWWNVLPFS IANPST-WVS HVTPFYQSGM QRIMDSTFYD
YP_003041132.1_Photorhabdus_as    NIVNFNDHYA SDWWNVLPFS IANPST-WVS HVTPFYQSGM QRIMDSVFYD
YP_003612281.1_Enterobacter_cl    NIVSFNDYYA GLTNDCKIFS IANMAS-WAG HSGQGYTDGA LRIMDSEIYD
ZP_05083622.1_Pseudovibrio_sp.    NIVIFNDWYD TPLFWDG-GG LLNLPS-WSA HLPFNYSTAF NAINASEFYS
YP_001280842.1_Psychrobacter_s    NVVLFNDVYA SPIWDVSLFS LLNIPTGWYA HINGITTDAI SRISNSTFYE
ZP_08387820.1_Sphingomonas_sp.    NLILFSDDYA NPAWPYGPFA LYNIPGGWAA HVAGITSDAV TRITQSAFYD
ZP_07775481.1_Pseudomonas_fluo    QMNRDSTIIV ANLSDPARAT TWVQDLNRNA EPHKGD---T FIIGSDGNDL
YP_002872716.1_Pseudomonas_flu    QMSRDATVIV ANLSDPARAN TWVQDLNRNA EPHKGN---T FIIGSDGDDL
BAA36468.1_Pseudomonas_fluores    LTSRDSTIIV ANLSDPARAN TWVQDLNRNA EPHKGN---T FIIGSDGNDL
YP_004354131.1_Pseudomonas_bra    LTEKDSTIIV ANLSDPARAN TWVQDLNRNA ETHTGS---T FIIGSDSNDL
YP_607820.1_Pseudomonas_entomo    WTTRDSTVVV SNLSDTTRGS TWVEDLNRNA EPHKGS---T FIIGTDKDDL
ZP_06189896.1_Serratia_odorife    LTNKDSTVIV SNLSEVTRGN TWVEDLNRNA EKHSGP---T FIIGSEGNDL
YP_001478438.1_Serratia_protea    LTGKDSTVIV SNLSDVTRGT TWVEDLNRNA EKHSGP---T FIVGSDSNDL
NP_929565.1_Photorhabdus_lumin    LTHKDSTIVV SNLSNVTREN TWVQDLNRNA EKHTGS---T FIIGTDGNDL
YP_003041132.1_Photorhabdus_as    LTHKDSTIVV SNLSNVTRGN TWVQDLNRNA EKHTGS---T FIIGTDGNDL
YP_003612281.1_Enterobacter_cl    FTHQNYNIIV SNLSEAYRST TWVSDLN-KS VTHVGS---T FIIGTETNDL
ZP_05083622.1_Pseudovibrio_sp.    EMNRDSVVII SGLSDQKRDD TWVEDIQIPL DPTGHHGQDA YILGSTSDDL
YP_001280842.1_Psychrobacter_s    YTKQDSTVIV ADLTAVSRAT TWVQDDK--S HTSSHYGSAA FIVGSEHNDL
ZP_08387820.1_Sphingomonas_sp.    LTSRDSLVIV SNLTAATRDI AWVEDLARPS DRHGHVGDSA FLIGSQFGDR
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Fig. 4: Multiple sequence alignment of amino acid residues of cold active lipases from 13 species (*P. fluorescens, P. brassicacearum, Serratia odorifera, S. proteamaculans, P. entomophila, Photorhabdus luminescens, Photorhabdus asymbiotica, Enterobacter cloacae, Pseudovibrio* sp., *Psychrobacter* sp., *Sphingomonas* sp.)

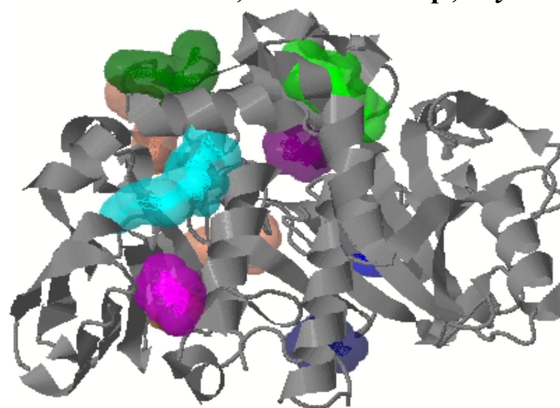

Fig. 5: Active site residues of predicted protein structure indicated by different colors, where cyan color indicates the site1.

Table 2: Energy values of the three inhibitors

Inhibitor	Formula	Energy value
P-Nitrophenol	$C_6H_5NO_3$	139.11 g/mol
Acetate ion	$C_3H_3O_2$	59.04 g/mol
Diethyl phosphonate	$C_4H_{11}O_3P$	138.1 g/mol

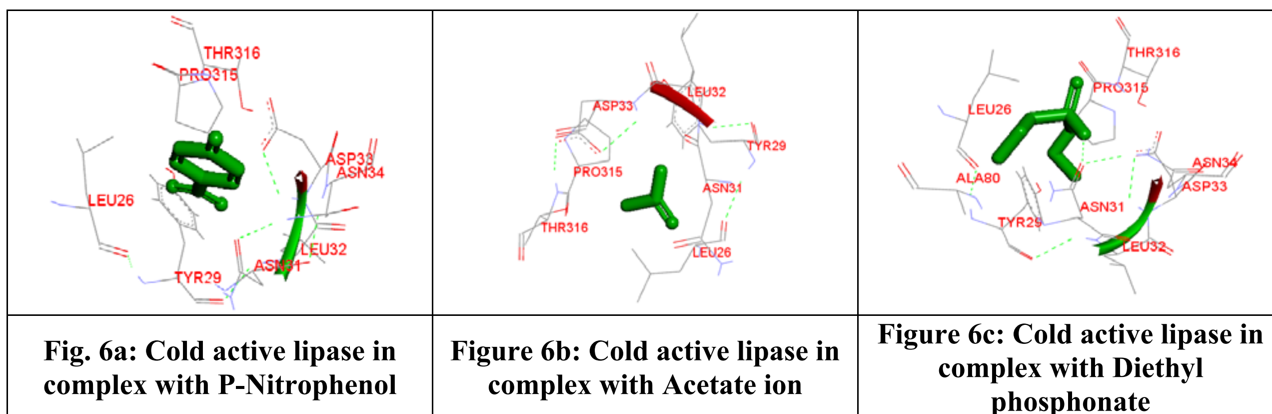

Fig. 6a: Cold active lipase in complex with P-Nitrophenol

Figure 6b: Cold active lipase in complex with Acetate ion

Figure 6c: Cold active lipase in complex with Diethyl phosphonate

Table 3: Hydrogen bonds along with their distances between the three inhibitors and active site residues of cold active lipase as deciphered using Discovery studio.

Inhibitor 1: P-Nitrophenol			
Cold active lipase		**Inhibitor 1**	**Distance (Å)**
Residue	**Atom**		
LEU 26	CB	C	3.07
TYR 29	CB	N	3.63
ASN 31	CG	C	2.88
ASP 33	OD1	N	3.16
ASN 34	CB	C	3.74
PRO 315	CG	N	3.67
THR 316	OG1	O	3.65
Inhibitor 2: Acetate ion			
Cold active lipase		**Inhibitor 2**	**Distance (Å)**
Residue	**Atom**		
LEU 26	CD2	C	3.24
TYR 29	CB	O	3.29
ASN 31	OD1	O	3.49
ASP 33	OD1	O	2.94
PRO 315	CB	C	3.51
THR 316	OG1	C	3.18
Inhibitor 3: Diethyl phosphonate			
Cold active lipase		**Inhibitor 3**	**Distance Å**
Residue	**Atom**		
LEU 26	CB	O	3.24
TYR 29	CB	C	3.28
ASN 31	OD1	H,O	1.95
ASP 33	OD1	O	3.26
ALA 80	CB	C	3.42
PRO 315	CG	C	3.86
THR 316	OG1	H	3.67

Docking of cold active lipase with potential inhibitors

Docking of cold active lipase was performed with three inhibitors namely p-Nitrophenol, Acetate ion and Diethyl phosphonate (Table 2). The three final docked conformations obtained for the different inhibitors were evaluated based on the number of hydrogen bonds formed and bond distance between atomic co-ordinates of the active site and inhibitor (Fig.6a-c). The energy values of the three inhibitors are 139.11, 59.04 and 138.1 g/mol respectively (Table 4.10). To evaluate the structural similarity of *P. fluorescens* cold active lipase with related bacterial species, we performed a multiple sequence alignment of *P. fluorescens* cold active lipase with the cold active lipase of species namely *Pseudomonas fluorescens, Pseudomonas brassicacearum, Serratia odorifera, Serratia proteamaculans, Pseudomonas entomophila, Photorhabdus luminescens, Photorhabdus asymbiotica, Enterobacter cloacae, Pseudovibrio* sp., *Psychrobacter* sp. and *Sphingomonas* sp.

The residues based on the results, and it was observed that the following stretches were highly conserved across species: Leu26, Tyr29, Asn31, Asp33, Asn34, Phe36, Ala37, Val38 Tyr40, Gln41, Ser287, Phe288, Asn289, Asp290, Ile311, Ser312, Leu314, Pro315, Thr316, Ala317, Asp320, Gly321, Arg324, Ala343, Asn344, Leu345, Ser346, and Asp347. Among these stretches, the following amino acids in *P. fluorescens* are involved in hydrogen bonding interactions with various inhibitors: Leu26, Tyr29, Asn31, Asp33, Pro315 and Thr316. The hydrogen bond interactions between the three inhibitors and cold active lipase along with their bond distances are shown in Table 3.

CONCLUSION

So with this *in silico* analysis it was concluded that *P. vancouverensis* lipase protein can also play similar role in lipid metabolic process and triglyceride lipase functional activity as reported for *P. fluorescens* lipase protein. Sequential and structural study of *P. fluorescens* lipase protein elucidate that *P. vancouverensis* lipase can have similar functional activity.

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REFERENCES

Alquati C, De Gioia L, Santarossa G, Alberghina L, Fantucci P, Lotti M (2002). The cold-active lipase of Pseudomonas fragi: Heterologous expression, biochemical characterization and molecular modeling. Eur J Biochem. 269:3321-3328.

Angkawidjaja C, Matsumura H, Koga Y, Takano K, Kanaya S. (2010). X-ray crystallographic and MD simulation studies on the mechanism of interfacial activation of a family I.3 lipase with two lids. J Mol Biol. 400(1):82-95.

Carriere F, Thirstrup K, Hjorth S. (1994). Cloning of the classical guinea pig pancreatic lipase and comparison with the lipase related protein. FEBS Lett. 388:63-68.

Case RJ, Boucher Y, Dahllo I, Holmstrom C, Doolittle WF, Kjelleberg S. (2007). Use of 16S rRNA and rpoB Genes as Molecular Markers for Microbial Ecology Studies. Appl Environ Microbiol. 73(1):278–288. doi: 10.1128/AEM.01177-06

Dieckelmann M, Johnson LA, Beacham IR (1998). The diversity of lipase from psychrotrophic strains of *Pseudomonas*: A novel lipase from a highly lipolytic strain of *Pseudomonas fluoroscens*. J Appl Microbiol. 85:527-536.

Georlette D, Blaise V, Collins T, Amico SD, Gratia E, Hoyoux A, Marx JC, Sonan G, Feller G Gerday C (2004). Some like it cold: biocatalysis at low temperatures. FEMS Microbiol Rev. 28:25-42.

Gerday C, Aittaleb M, Bentahir M, Chessa JP, Claverie P, Collins T, D'Amico S, Dumont J, Garsoux G, Georlette D, Hoyoux A, Lonhienne T, Meuwis M, Feller G (2000). Cold adapted enzymes: from fundamentals to biotechnology. Trend Biotechnol. 18:103-107.

Gomes J, Steiner W (2004). The biocatalytic potential of extremophiles and extremozymes. Food Technol Biotechnol. 42:223-235.

Gupta GN and Prakash V (2014). Isolation and identification of a novel, cold active lipase producing psychrophilic bacterium *Pseudomonas vancouverensis*. Trend Biosci. Nov. 7(22): In Press.

Joseph B (2006). Isolation, purification and characterization of cold adapted extracellular lipases from psychrotrophic bacteria: Feasibility as laundry detergent additive. Ph.D. Thesis. Allahabad Agricultural Institute– Deemed University, Allahabad, India.

Joseph B, Ramteke, PW, Thomas G, Shrivastava N (2007). Standard Review Cold-active microbial Lipases: a versatile tool for industrial applications. Biotechnol Mol Biol Rev. 2(2):039-048.

Kitchen DB, Decornez H, Furr JR, Bajorath J (2004). "Docking and scoring in virtual screening for drug discovery: methods and applications". Nat rev Drug discov. 3(11):935–49.doi:10.1038/nrd1549.PMID 15520816.

Kumar V, Kumar CS, Hari G, Venugopal NK, Vijendra PD Basappa GB (2013). Homology modeling and docking studies on oxidosqualene cyclases associated with primary and secondary metabolism of *Centella asiatica*. Springer Plus. 2:189-200.

Lee HK, Min JA, Sung HK, Won HS, Byeong CJ (2003). Purification and Characterization of Cold Active Lipase from Psychrotrophic *Aeromonas* sp. LPB4. J Microbiol. 41:22-27.

Narinx E, Baise E, Gerday C (1997). Subtilisin from Psychrophilic Antarctic bacteria: characterization and site directed mutagenesis of residues possible involved in the adaptation to cold. Prot Eng. 10:1271-1279.

Wintrode PL, Miyazaki K Arnold FH (2000). Cold adaptation of a mesophilic subtilisin-like protease by laboratory evolution. J Biol Chem. 275:31635–31640.

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