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Original Article

A Bioinformatics Approach for Identification of Micro-organism Showing Highest Homology for Lipase B Gene

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ABSTRACT

Lipase-B from *Candida antarctica* is used as effective biocatalyst in various industries mainly in the preparation of drugs like S-Pregabalin. But the production of this enzyme from this organism is very difficult for Indian conditions. In the present study, a putative gene was identified with the help of bioinformatics. Results obtained showed Sporisorium *reilianum srz2* lipase B gene 75% homology with *Candida antarctica* lipase B (CALB) gene. Docking with substrate (IBG-Di-Methyl ester) also proved to be very efficient. The two organisms were having similar structure and function of industrial important enzyme Lipase B. The analysis proved to be significant as lipase B can be produced from non-pathogenic microorganism in a cost effective method.

Keywords: *Sporisorium reilianum srz2, Candida antarctica*, lipase B gene.

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INTRODUCTION

Lipases have emerged as one of the leading biocatalysts with proven potential for contributing to the multibillion dollar underexploited lipid technology bio-industry and have been used in *in situ* lipid metabolism and *ex situ* multifaceted industrial applications [1]. Gupta and his coworkers (2007) suggested that lipases have ability to perform very specific chemical transformation (biotransformation) which has made them increasingly popular in the food, detergent, cosmetic, organic synthesis, and pharmaceutical industries [2]. Currently, lipase B is the widely targeted enzyme for protein engineering so as to improve and optimize its substrate specificity and enantioselectivity [3].

Lipase-B from *Candida antarctica* is used as effective biocatalyst in various industries (one of its application is in the preparation of drugs like S-Pregabalin, which is used to relieve the neuropathic pain and epilepsy). *Candida antarctica* grows at very low temperature; the cultivation is very difficult for Indian conditions. As *Candida antarctica* Lipase B (CALB) expression levels in the native organism are too low, which needs a recombinant over-expression for the large-scale production of these biocatalysts [4]. As the lipase B gene sequence is already patented [1] the best alternative is use of a bioinformatics approach to "mine" data and extract relevant biological information from the vast amount of biological data available in public databases. The technique is basically utilized to identify genes of interest and subject them to various *In-silico* analyses. Such information explores novel insights with respect to the genes in question and opens up novel arena for further research in the field of scientific world. The potential of the present bioinformatics approach aims to screen/ identify microorganism with the similar Lipase B gene which exhibits superior enzyme activity.

MATERIALS AND METHODS

Identification of organism through bioinformatics approach: Using bioinformatics approach homologs was searched for CALB genome which was later compared with *Sporisorium reilianum SRZ2* strain that was identified as a homolog through following steps:-

Protein sequence of CALB with UniProt ID 1TCA was retrieved from UniProtKB database (in FASTA format) which was uploaded as query sequence in Basic Local Alignment search tool (BLAST) programme using BLAST Protein(P) tool with default parameters like Max Identity, E-value, Max score. The protein sequence of *Sporizorium reilianum srz2* was reflected to be homologous with 75% identical to the query sequence. The protein sequence of *Sporizorium reilianum srz2* lipase B was retrieved from UniProtKB database with UniProt ID E6ZUC1 in FASTA format. Similarity sequence identification (Homology) was carried out between these two sequences through EMBOSS water, using Smith-Waterman alignment algorithm for attaining the local alignment analysis. The protein sequence of *Sporizorium reilianum srz2* lipase B was uploaded in **ProtParam** tool for attaining its physical and chemical parameters with its default parameters. The results and data about molecular weight, isoelectric point, half-life, amino acid composition, atomic formula values were observed.

Homology modeling: The 3dimensional (D) structure of *Sporizorium reilianum srz2* lipase B was predicted by homology modelling using Modeller 9.15 tool as its 3D structures was not reported at Protein Data Bank (PDB). The protein sequence *Sporizorium reilianum srz2* lipase B that was retrieved from UniProtKB database with ID E6ZUC1 possesses 341 amino acid (aa) length. The PDB templates required for homology modeling of *Sporizorium reilianum srz2* lipase B are retrieved from BLASTP tool against PDB database. The resulted templates were:

1. 4K6H_A (Chain A, Crystal Structure Of Calb Mutant L278m From Candida Antarctica)
2. 4K6G_A (Select seq pdb|4K6G|A Chain A, Crystal Structure Of Calb From Candida Antarctica)
3. 1TCB_A(Chain A, The Sequence, Crystal Structure Determination And Refinement Of Two Crystal Forms Of Lipase B From Candida Antarctica)

Active site docking studies using online and offline molecular docking tools. The top binding/active site for docking of the predictable lead molecules against Sporizorium *reilianum srz2* lipase B for each identified domain was predicted through RaptorX Binding online server. The predicted lead/drug molecules and the substrate IBG-Di-Methyl ester were retrieved from NCBI PubChem database. These compounds were docked against Sporizorium *reilianum srz2* lipase B protein using Autodock4.2 molecular docking tool to find out the potential lead/drug molecule [5].

RESULTS

The protein sequence of *Candida antarctica* lipase B was retrieved from UniProtKB database with UniProt ID 1TCA possesses 342 amino acid (aa) length.

```
        10         20         30         40         50
MKLLSLTGVA GVLATCVAAT PLVKRLPSGS DPAFSQPKSV LDAGLTCQGA
        60         70         80         90        100
SPSSVSKPIL LVPGTGTTGP QSFDSNWIPL STQLGYTPCW ISPPPFMLND
       110        120        130        140        150
TQVNTEYMVN AITALYAGSG NNKLPVLTWS QGGLVAQWGL TFFPSIRSKV
       160        170        180        190        200
DRLMAFAPDY KGTVLAGPLD ALAVSAPSVW QQTTGSALTT ALRNAGGLTQ
       210        220        230        240        250
IVPTTNLYSA TDEIVQPQVS NSPLDSSYLF NGKNVQAQAV CGPLFVIDHA
       260        270        280        290        300
GSLTSQFSYV VGRSALRSTT GQARSADYGI TDCNPLPAND LTPEQKVAAA
       310        320        330        340
ALLAPAAAAI VAGPKQNCEP DLMPYARPFA VGKRTCSGIV TP
```

The protein sequence *Sporizorium reilianum srz2* lipase B that was retrieved from UniProtKB database with ID E6ZUC1 possesses 341 amino acid (aa) length.

```
        10         20         30         40         50
MKFLTALTVL ASCSALASAT PLVKRLPSGS DPAYTLSKAQ LDSVLACQNG
        60         70         80         90        100
SPSSQKNPIL LVPGTGTTGP QSFDSNWIPL STQLGYSPCW VSPPPFMLND
       110        120        130        140        150
TQVNAEYIVN AVKVLSSASG AKVPVLTWSQ GGLAAQWALT FFPSIRTQVD
       160        170        180        190        200
RLMAFAPDYK GTVLAAFLTT PGLASESVWQ QQAGSALTTA LANAGGLTKI
       210        220        230        240        250
VPTTNLYSAT DDIVQPQTFN GPLDSGYLNG GAKNIQAQSV CGPLFVVDHA
       260        270        280        290        300
GTLTSQFSFV VGRSALRSTT GQAQSKDYGV TDCNPLPADS LTPDQKLRAE
       310        320        330        340
GLLLVAGANV AAGPKQNCEP DLMPYARQYA VGKRTCSGVI L
```

EMBOSS Water – Alignment:

Pair wise sequence alignment was carried out using EMBOSS Water – Alignment online server to find out the local alignments, gaps, matches and mismatches with the default parameters. **Water** uses the Smith-Waterman algorithm (modified for speed enhancements) to calculate the local alignment of a sequence to one or more other sequences. The gap insertion penalty, gap extension penalty and substitution matrix used to calculate the alignments are specified. Dynamic programming methods ensure the optimal local alignment by exploring all possible alignments and choosing the best. It does this by reading in a scoring matrix that contains values for every possible residue or nucleotide match. **Water** finds an alignment with the maximum possible score where the score of an alignment is equal to the sum of the matches taken from the scoring matrix. The result predicts an acceptable amount of similarity between both the sequences. The vertical lines denotes the identity, the dots represents the similarity and the horizontal lines represents the gaps (**Fig 1**).

- **Alignment**

```
########################################
# Program: water
# Rundate:            Sat 28 Jan 2012 10:36:53
# Commandline: water
#    -auto
#    -asequence /var/lib/emboss-explorer/output/624967/.asequence
#    -bsequence /var/lib/emboss-explorer/output/624967/.bsequence
#    -gapopen 10.0
#    -gapextend 0.5
#    -brief
#    -outfile outfile
#    -aformat3 srspair
# Align_format: srspair
# Report_file: outfile
########################################
```

- Align_format: pair

Report_file: stdout
Aligned_sequences: 2
1: CAA83122.1
2: CBQ70828.1
Length: 345
Score: 1312.5

```
#=======================================
#
# Aligned_sequences: 2
# 1:
# 2:
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 345
# Identity:     255/345 (73.9%)
# Similarity:   288/345 (83.5%)
# Gaps:          10/345 ( 2.9%)
# Score: 1312.5
#
#
#=======================================
```

```
  1 MKLL-SLTGVAGVLATCVA---ATPLVKRLPSGSDPAFSQPKSVLDAGLT     46
    ||.| :||    |||:|.|   |||||||||||||::..|:.||:.|.
  1 MKFLTALT----VLASCSALASATPLVKRLPSGSDPAYTLSKAQLDSVLA     46

 47 CQGASPSSVSKPILLVPGTGTTGPQSFDSNWIPLSTQLGYTPCWISPPPF     96
    ||..||||...|||||||||||||||||||||||||:|||:|||||
 47 CQNGSPSSQKNPILLVPGTGTTGPQSFDSNWIPLSTQLGYSPCWVSPPPF     96

 97 MLNDTQVNTEYMVNAITALYAGSGNNKLPVLTWSQGGLVAQWGLTFFPSI    146
    ||||||||.||:|||:..|.:.|| .|:||||||||||.|||.|||||||
 97 MLNDTQVNAEYIVNAVKVLSSASG-AKVPVLTWSQGGLAAQWALTFFPSI    145

147 RSKVDRLMAFAPDYKGTVLAGPLDALAVSAPSVWQQTTGSALTTALRNAG    196
    |::|||||||||||||||..|....:::.|||||..||||||||.|||
146 RTQVDRLMAFAPDYKGTVLAAFLTTPGLASESVWQQQAGSALTTALANAG    195

197 GLTQIVPTTNLYSATDEIVQPQVSNSPLDSSYLFNG-KNVQAQAVCGPLF    245
    |||:||||||||||||:|||||..|.||||.||..| ||:|||:||||||
196 GLTKIVPTTNLYSATDDIVQPQTFNGPLDSGYLNGGAKNIQAQSVCGPLF    245

246 VIDHAGSLTSQFSYVVGRSALRSTTGQARSADYGITDCNPLPANDLTPEQ    295
    |:||||:||||||:|||||||||||||||:|.|||:||||||||:.|||:|
246 VVDHAGTLTSQFSFVVGRSALRSTTGQAQSKDYGVTDCNPLPADSLTPDQ    295

296 KVAAAALLAPAAAAIVAGPKQNCEPDLMPYARPFAVGKRTCSGIV    340
    |:.|..||..|.|.:.|||||||||||||||.:|||||||||::
296 KLRAEGLLLVAGANVAAGPKQNCEPDLMPYARQYAVGKRTCSGVI    340
```

Fig 1: EMBOSS Water – Alignment of *Candida antarctica* and *Sporisoriumreilianum SRZ2* lipase B

Physical and chemical parameters of protein sequence of *Candida antarctica* and *Sporizorium reilianum srz2* lipase B using ProtParam tool

ProtParam results of *Candida antarctica*

Number of amino acids: 342; **Molecular weight:** 35517.57; **Theoretical pI:** 8.12

Amino acid composition:
Ala (A) 40:11.7%; Arg (R) 9: 2.6%; Asn (N) 14:4.1%; Asp (D) 14: 4.1%; Cys (C) 7: 2.0%; Gln (Q) 18: 5.3%; Glu (E) 4: 1.2%; Gly (G) 28: 8.2%; His (H) 1: 0.3%; Ile (I) 11: 3.2%; **Leu (L) 36: 10.5%;** Lys (K) 11: 3.2%; Met (M) 5 :1.5%; Phe (F) 10: 2.9%; Pro (P) 31: 9.1%; Ser (S) 32: 9.4%; Thr (T) 30: 8.8%; Trp (W) 5: 1.5%; Tyr (Y) 9: 2.6%; Val (V) 27: 7.9%; Pyl (O) 0; 0.0%; Sec (U) 0; 0.0%
(B) 0 0.0%
(Z) 0 0.0%
(X) 0 0.0%
Total number of negatively charged residues (Asp + Glu): 18
Total number of positively charged residues (Arg + Lys): 20

Atomic composition:

Carbon	C	1584
Hydrogen	H	2507
Nitrogen	N	419
Oxygen	O	482
Sulfur	S	12

Formula: $C_{1584}H_{2507}N_{419}O_{482}S_{12}$
Total number of atoms: 5004

Extinction coefficients:

Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.

Ext. coefficient 41285
Abs 0.1% (=1 g/l) 1.162, assuming all pairs of Cys residues form cystines

Ext. coefficient 40910
Abs 0.1% (=1 g/l) 1.152, assuming all Cys residues are reduced

Estimated half-life:

The N-terminal of the sequence considered is M (Met).

The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).
>20 hours (yeast, in vivo).
>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 40.25
This classifies the protein as unstable.

Aliphatic index: 88.19

Grand average of hydropathicity (GRAVY): 0.121

Based on the results of the sequence homology the Probable Lipase B precursor [*Sporisoriumreilianum SRZ2*] was selected for unwinding its Physical and chemical parameters of its protein sequence
Number of amino acids: 341
Molecular weight: 35591.50
Theoretical pI: 8.11
Amino acid Composition:
Ala (A) 39: 11.4%, Arg (R) 8: 2.3%, Asn (N) 14: 4.1%, Asp (D) 15: 4.4%, Cys (C) 7: 2.1%, Gln (Q) 22: 6.5%, Glu (E) 4 : 1.2%, Gly (G) 28: 8.2%, His (H) 1: 0.3%, Ile (I) 8: 2.3%, **Leu (L) 39: 11.4%,** Lys (K) 13: 3.8%, Met (M) 4: 1.2%, Phe (F) 11: 3.2%,Pro (P) 26: 7.6%, Ser (S) 33: 9.7%, Thr (T) 29: 8.5%, Trp (W) 5: 1.5%, Tyr (Y) 9: 2.6%, Val (V) 26: 7.6%, Pyl (O) 0: 0.0%, Sec (U) 0 0.0%
(B) 0 0.0%
(Z) 0 0.0%
(X) 0 0.0%
Total number of negatively charged residues (Asp + Glu): 19
Total number of positively charged residues (Arg + Lys): 21
Atomic composition:
Carbon C 1584
Hydrogen H 2505
Nitrogen N 421
Oxygen O 487
Sulfur S 11
Formula: $C_{1584}H_{2505}N_{421}O_{487}S_{11}$
Total number of atoms: 5008

Extinction coefficients:
Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.
Ext. coefficient 41285
Abs 0.1% (=1 g/l) 1.160, assuming all pairs of Cys residues form cystines
Ext. coefficient 40910
Abs 0.1% (=1 g/l) 1.149, assuming all Cys residues are reduced

Estimated half-life:
The N-terminal of the sequence considered is M (Met).
The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).
>20 hours (yeast, in vivo).
>10 hours (Escherichia coli, in vivo).

Instability index:
The instability index (II) is computed to be 39.16
This classifies the protein as stable.

Aliphatic index: 87.30

Grand average of hydropathicity (GRAVY): 0.063 Comparative study of Physical and chemical parameters of both the protein sequence reflected a negligible amount of difference between *Candida antarctica* and *Sporisoriumreilianum SRZ2* lipase B. Both the proteins reflected a similar kind of amino acid composition, equal number of carbon atom composition with negligible difference in the amount of Hydrogen, nitrogen, oxygen and sulfur. Similarly there was also a negligible amount of difference in Instability index, aliphatic index and Grand average of hydropathicity (GRAVY).

Homology modeling of *Sporizorium reilianum srz2* lipase B

The 3dimensional (D) structure of *Sporizorium reilianum srz2* lipase B was predicted by homology modelling using Modeller 9.15 tool as its 3D structures was not reported at Protein Data Bank (PDB) (Fig 2).

Model refinement, evaluation and Structure Validation

Structure validation of the modelled structure (Fig2) was carried using various web servers like WhatIF (http://swift.cmbi.ru.nl/servers/html/index.html), PROCHECK for Ramachandran plot analysis. Stereochemical quality and accuracy of the selected models was further improved by subjecting it to energy minimization with the GROMOS 96 43B1 parameters set, implementation of Swiss-PDB Viewer. Validation of generated models was further performed by VERIFY 3D and ERRAT programs. ProSA was used for the analysis of Z scores and energy plots. The results predicted 16 beta pleated sheets and 7 alpha helices. The Z score was Z-Score: -2.488 and the Qmean score was 0.562. The Structural assessment was carried out through Ramchandran plot (Fig 3) and ProFunc(Fig4).

Fig 2: Homology modeling of *Sporizorium reilianum srz* Lipase B

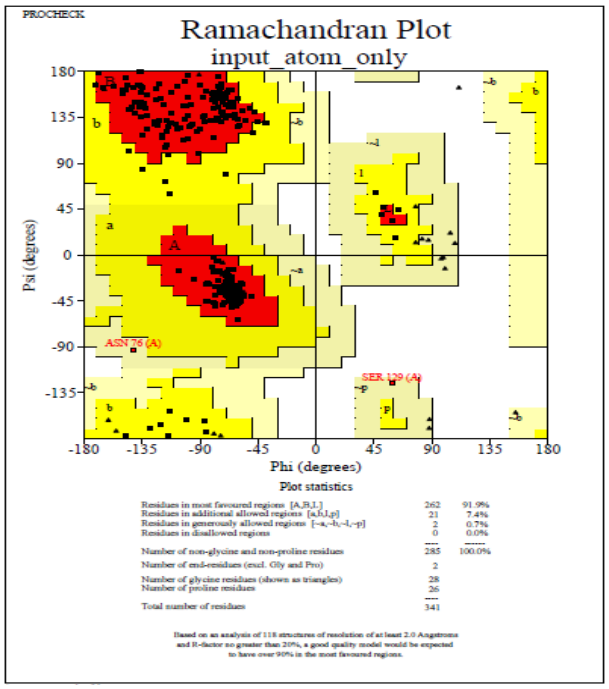

Fig 3: Ramchandran plot of *Sporizorium reilianum srz2* lipase B

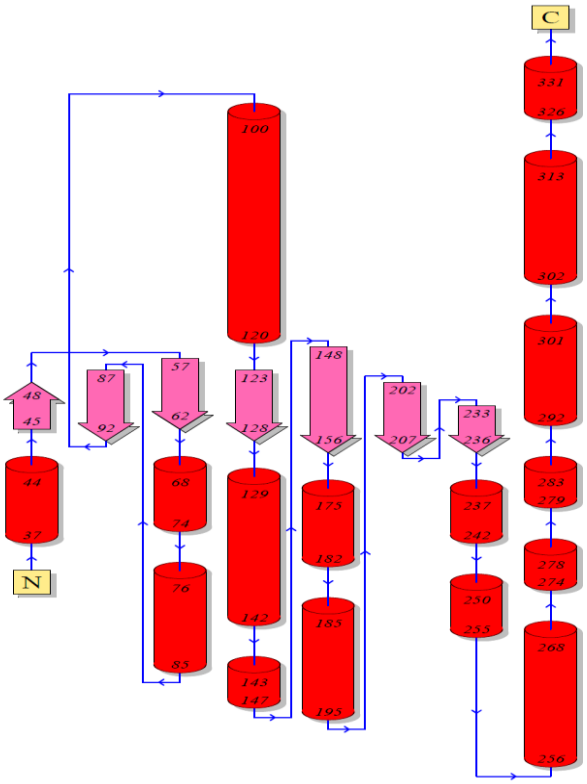

Fig 4: Structural characterization of *Sporizorium reilianum srz2* lipase B using ProFunc

Active site docking studies using online molecular docking tools.

The top binding/active site for docking and the anticipated lead molecules of Sporizorium *reilianum srz2* lipase B for each identified domain were predicted through RaptorX Binding online server (Fig 5). The results reflects the following:

Candida Antarctica (1TCB)

Serine-130, Aspergine-212, Histidine-249

Sporisorium active site (3ICV)

Glutamine-130, Aspergine-212, Histidine-249

Lipase-B Specifications

Name: Lipase B

Synonym: Triacylglycerol hydrolase

Protein name: EC=**3.1.1.3**

Source: *Candida antarctica* (yeast) (*Trichosporonoryzae*)

Molwt: 35 kDa; pH value:5.0 - 7.0; Isoelectric point : 6.0

Protein attributes

Sequence annotation (features)

Feature key	Positions	Length	Description	Graphical view	Feature identifier
Molecule processing					
Signal peptide	1 – 18	18	Potential		
Propeptide	19-25	7			PRO_0000021595
Chain	26-342	317	Lipase B		PRO_0000021596
Sites					
Active site	130	1			
Active site	212	1			
Active site	249	1			
Amino acid modifications					
Glycosylation	99	1	N-linked (GlcNAc...)		
Disulfide bond	47-89				
Disulfide bond	241-283				
Disulfide bond	318-336				

The number of amino acids, molecular weight and theoretical isoelectric point of *C. Antarctica* lipase B is 342 a.a, 35 kDa and 6 respectively while that of *Sporisorium reilianum* lipase B is 341 a.a., 35.6 kDa and 8 respectively. The anticipated lead molecules for the modeled protein *Sporisorium reilianum* lipase B predicted by RaptorX Binding server is as under:

Top binding sites for sequence segment [6]:

1) Binding residues: G64 T65 W128 S129 Q130 D158 T162 L164 A165 V178 Q181 I213 V214 H249 L303 A306
ligands: MPD, HEE, CL, PO4, 1R1
2) Binding residues: T35 P94 N99 D100 V103
ligands: NAG
3) Binding residues: G64 T67 Q71 S72 W128 S129 L302
ligands: CL, GOL, EPE, NH4, PO4
4) Binding residues: T65 Q181 D212 I213 L303 L304 V310
ligands: PE8, BOG

The PubChem Ids of the predicted ligands that were found to act as potential drug like was 5288834 for (4S)-2-METHYL-2,4-PENTANEDIOL (MPD), 439174 for N-Acetyl-D-Glucosamine (NAG) and 78798 for Octaethylene Glycol (PE8). The PubChem Id of the substrate is 15152901 for 3-Isobutylglutaric acid dimethyl ester (IBG-Di-Methyl ester).

The results reflect differences in the binding sites for various lead molecules (Table 1). This may due it's the efficacy of amino acids for contribution in binding pocket.

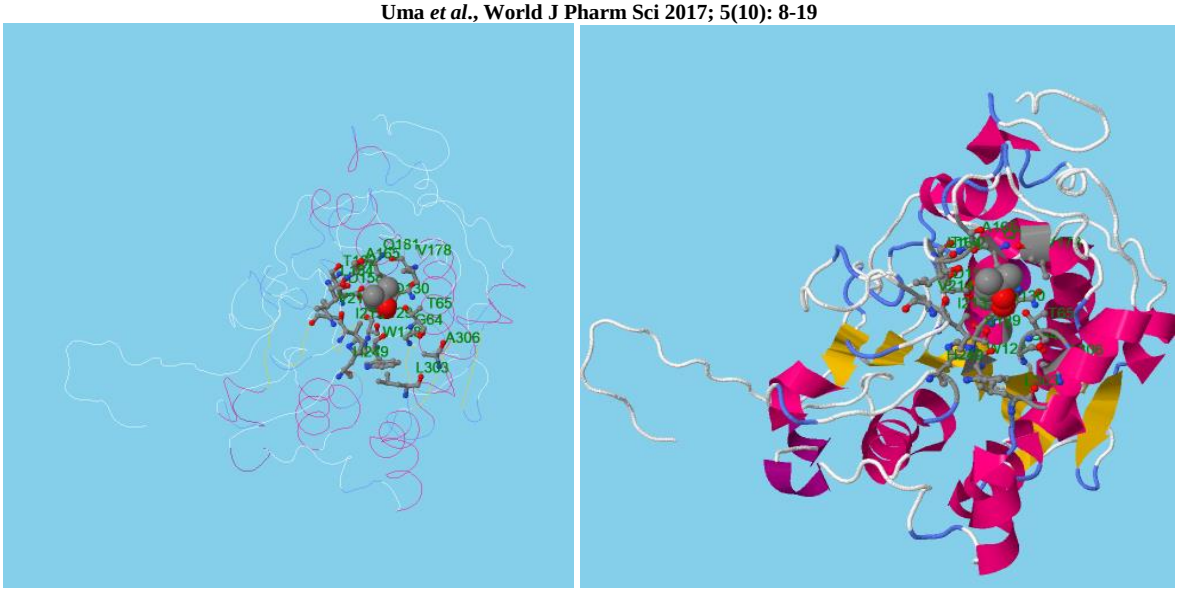

Fig 5: The active/binding pocket of protein *Sporisorium reilianum* lipase B

The molecular docking predicted N-Acetyl-D-Glucosamine (NAG) to be the potential ligand/drug molecule with reference its binding energy (-3.52 Kcal/mol), ligand efficiency (-0.23) and inhibition constant(2.62) in comparison to the to the other predicted lead molecules. It pursues hydrogen bonding with Thr35, Asp100, Glu176 which reflects its tough binding affinity **(Fig 6**).

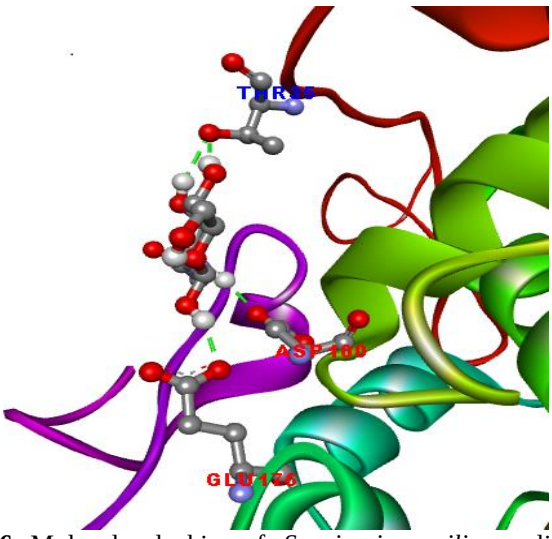

Fig6: Molecular docking of *Sporisorium reilianum* lipase B and N-Acetyl-D-Glucosamine (NAG) interaction complex

Docking of the substrate, 3-Isobutylglutaric acid dimethyl ester **(**IBG-Di-Methyl ester) against *Sporisorium reilianum* lipase B (**Fig7**)predicted better scores in comparison with N-Acetyl-D-Glucosamine (NAG) based on its binding energy (-4.27 Kcal/mol), ligand efficiency (-0.28), inhibition constant(742.74) and electrostatic energy (-0.14). It pursues hydrogen bonding with Thr65, Ser129, Gln181 and Val178, which reflects its tough binding affinity.

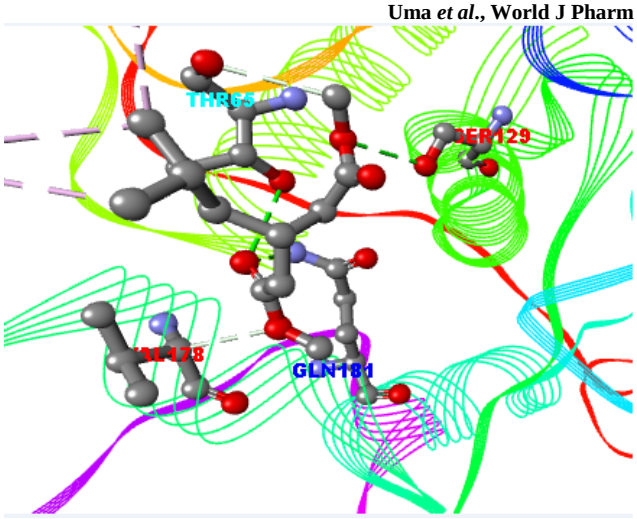

Fig7: Molecular docking of 3-Isobutylglutaric acid dimethyl ester **(**IBG-Di-Methyl ester) against *Sporisorium reilianum* lipase B.

Table1: Molecular docking of *Sporizorium reilianum srz2* lipase B with different ligands.

Target protein	Ligand /drug	Binding Energy (kcal/mol)	Ligand Efficiency (kcal/mol)	Inhibition Constant	Electrostatic energy	Hydrogen Bond	Electrostatic interaction	Hydrophobic interaction
Sporizorium reilianum srz2 lipase B	(4S)-2-Methyl-2,4-Pentanediol (MPD)	-3.03	-0.38	6.0	-0.11	Thr65, Ser129	Nil	Nil
-Do-	N-Acetyl-D-Glucosamine (NAG).	-3.52	-0.23	2.62	-0.41	Thr35, Asp100,Glu176	Nil	Nil
-Do-	Octaethylene Glycol	-2.95	-0.41	1.19	-0.02	Nil	Nil	Nil
-Do-	3-Isobutylglutaric acid dimethyl ester (IBG-Di-Methyl ester)	-4.27	-0.28	742.74	-0.14	Thr65, Ser129, Gln181 and Val178	Nil	Ala306 , Val310

DISCUSSION: Uppenberg and his coworkers worked on the DNA and amino acid sequence of *C. Antarctica* lipase B. They stated that primary sequence had no homology with the sequences of other known lipase and also stated that the enzyme has a Ser-His-Asp catalytic triad in its active site [7]. The result of the present study proves that at active site of *Sporisorium reilianum* lipase B has glutamine instead of serine. So catalytic triad is represented as Gln-His-Asp active site. Both the amino acids are polar in nature, forms hydrogen bonds which participates as proton donor or acceptors. CALB structure appears to be in an 'open' conformation with a rather restricted entrance to the active site [7]. This lipase B conformation accounts for the greater substrate specificity and high degree of stereo specificity. Since *Sporisorium reilianum srz2* lipase B gene showed 75% similarity to CALB gene in motif and docking with substrate (IBG-Di-Methyl ester),

proves that *Sporisorium reilianum srz2* lipase B will also have similar degree of activity.

CONCLUSION: Putative gene identification with the help of bioinformatics approach reflected similarity sequence identification, motif search and active site docking studies of lipase-b protein in different organisms proved that one of the organisms *Sporisorium reilianum srz2* lipase B gene showed 75% similarity in motif and docking with substrate (IBG-Di-Methyl ester) when compared with *Candida antarctica* lipase B gene.

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REFERENCES

1. Joseph B et al. Cold active microbial lipases: Some hot issues and recent developments. Biotechnology Advances 2008; 26: 457–70.
2. Gupta N et al. Alkaline lipase from a novel strain *Burkholderia multivorans*: Statistical medium optimization and production in a bioreactor. Process Biochemistry 2007; 42(2): 518–26.
3. Lutz S. Engineering lipase B from *Candida antarctica*. Tetrahedron: Asymmetry 2004; 15(18): 2743-8.
4. Poojari Y, Clarson SJ. Thermal stability of Candida antarctica lipase B immobilized on macroporous acrylic resin particles in organic media. Biocatalysis and Agricultural Biotechnology 2013; 2(1): 7-11.
5. Morten Källberg, et al. Template-based protein structure modeling using the RaptorX web server. Nature Protocols 2012; 7: 1511-22.
6. Raza S et al. Enantioselectivity in Candida antarctica lipase B: A molecular dynamics study. Protein Science : A Publication of the Protein Society 2001; 10(2): 329-38.
7. Uppenberg J et al. The sequence, crystal structure determination and refinement of two crystal forms of lipase B from Candida antarctica. Structure 1994; 2(4): 293-08.