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# Molecular Modelling and Structure analysis of S-ribosyl homocysteinase from *Aeromonas hydrophila*

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**In the Present study, Molecular modelling and structure analysis was performed for S-ribosyl homocysteinase from *Aeromonas hydrophila.* This is to determine new set of compounds that can be explored as antibiotics sensing and targeting the Quorum sensing proteins. The Probable ligands and their binding sites were also determined using ligprof server. HPS[2- Amino- 4- Mercapto- Butyric acid] was found to be the best ligand which can bind to the 3D structure of the target protein.**

**Key words:** *Aeromonas hydrophila, Quorum sensing,* S-ribosyl homocysteinase, AI-2 synthase, Insilico analysis.

In humans, *Aeromonas hydrophila* infections are known to cause gastroenteritis and wound infections. Investigations for developing a potential vaccine for its control are underway. Aeromonas hydrophila are ubiquitous, facultative anaerobe, gram-negative bacteria found in fresh, brackish, marine, chlorinated and non-chlorinated water supplies worldwide (Janda 1991, Kaper *et al* 1980, and Vander Kooj *et al* 1988). In recent years, a sharp increase in the acute diarrohoeal incidences in human by A. hydrophila has generated a great interest in Aeromonas sp. A. hydrophila is an emergent human pathogen which caused serious health problem regularly around the globe (Janda and Abbott 1998; Abbott et al. 1998; Joseph and Carnahan 2000). The most widely used method for controlling A. hydrophila infections in aquaculture is using antimicrobial drugs. Extensive use of antibiotic has resulted in rapid spread of multi-drug resistant pathogens (Rathore *et al*, 2006). There is an essential for controlling A. hydrophila infection using different antibiotics which is targeted the speciûc protein/ enzyme of A. hydrophila.

Today, there is in an urgent need for novel antibacterial drugs, as many important human pathogens have acquired multiple antibiotic resistance factors. Recently, it has been suggested to develop therapeutics that attack bacterial virulence rather than kill bacteria. Such drugs are called “antipathogenic” and are believed to reduce the development of antibiotic resistance. Specifically, cell-density-dependent gene regulation (quorum-sensing) in bacteria has been proposed as a potential target. While promising

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reports exist about quorum-sensing blockers in gram-negative bacteria, the use of the quorum-sensing system as a drug target is now seen in an increasingly critical way. Furthermore, down-regulation or mutation of the quorum-sensing system increases bacterial persistence in device-related infection, suggesting that interference with quorum-sensing would enhance rather than suppress this important type of disease. Targeting quorum-sensing systems might in principle constitute a reasonable way to find novel antibacterial drugs. Quorum sensing is a process of bacterial communication that relies on the production, detection, and response to extracellular signaling molecules called autoinducers (Fuqua and Greenberg, 2002; Fuqua and Winans, 1994; Waters and Bassler, 2005).

Gram-negative bacteria commonly use acyl-homoserine lactone molecules (AHLs) as autoinducers (Bassler, 1999; de Kievit and Iglewski, 2000; Fuqua et al., 1996). In Gram-negative bacteria *Aeromonas hydrophila*, quorum sensing typically involves the production, release and detection of acylated homoserine lactone signalling molecules called autoinducers. LuxS is the AI-2 synthase and that AI-2 is produced from S-adenosylmethionine in three enzymatic steps. The substrate for LuxS is S-ribosylhomocysteine, which is cleaved to form two products, one of which is homocysteine, and the other is AI-2. The LuxS is an autoinducer synthase produced by many diverse bacteria, including both Gram-negative and Gram-positive species. The gene *luxS* encodes an S-ribosyl homocysteinase, which converts S-ribosyl homocysteine from the methyl cycle into homocysteine (which is then returned to the active methyl cycle), as well as the autoinducer 2, a borate diester of the cyclized 4,5-dihydroxy-2,3-pentanedione (Schauder and Bassler 2001, Schauder *et al* 2001, Winzer *et al* 2002, Surette *et al* 1999 and Lerat *et al* 2004).

The AI-2 system has been shown to regulate several physiological activities including pathogenicity (through the control of toxins and other virulence expression factors), motility, biofilm formation, antibiotic production, and bioluminescence, among others. Thus, AI-2 involves mainly in the bacterial chemotaxis and motility and exerts its effect on both quorum sensing and pathogenesis. This study aims in the indirect inhibition of the synthesis and secretion of autoinducer proteins by directly targeting inhibitors against LuxS coding S-ribosyl homocysteinase in *Aeromonas hydrophila*, a Gram negative bacterium.

The ability of *insilico* tools to predict the three dimensional protein structures give ways for identifying potential drugs targeting against quorum sensing pathway in *Aeromonas hydrophila*, a heterotrophic, gram-negative bacterium. The thorough knowledge of 3D structure of proteins is necessary to analyse the binding mode of its specific substrate as well as inhibitors. Since, there was no 3D structure of S-ribosyl homocysteinase in *Aeromonas hydrophila* available with the Protein Data Bank, the current project involves determination of 3D structure of the target by Homology modeling using Modeller and *insilico* analysis of binding site and structural alignment and orientation in the target organism.

Methodology

**Steps involved**

1. Retrieval of the Target Sequence from the GenBank- Gene repository database [http://www.ncbi.nlm.nih.gov/gene].
2. The Target sequence in FASTA format is submitted to BLAST search for homologous sequences with known structures in Pdb [Bolton *et al*, https://pubchem.ncbi.nlm.nih.gov/].
3. The homologous sequence with known structure in Pdb is taken as template and the 3D structure of the target protein was predicted by Homology modeling using Modeller 9v2 ( Sali & Blundell, 1993, https://salilab.org).
4. The best predicted structure among the possible models were verified using Structure validation server- Procheck (Laskowski *et al* 1993).
5. The structure – structure alignment between the predicted 3D structure and the template was analysed using DALI server ( Holm and Rosenström 2010 ).
6. The possible binding ligands within the active site of the target Protein was determined using Ligprof server ( Grzegorz Koczyk *et al*, 2007).

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## RESULTS AND DISCUSSION

### Sequence retrieval

The target protein sequence with accession number ACA61284 was retrieved from NCBI –Genbank database. The sequence was converted to FASTA format and submitted to Blast search. With all the default parameters of BLAST, the homologous sequence was searched against PDB database. Even though it returned several bacterial Homocysteinase homologs, the sequence of 'A' chain of Crystal Structure of Autoinducer-2 Production Protein (LuxS) from *Heamophilus influenzae* was selected as the template for doing comparitive modeling using Modeller. The template sequence shows 73% identity with no gaps with the target and it has it's 3D structure deposited with Protein data Bank 1JOE ( Figure 1).

### FASTA sequence

Target: S-ribosylhomocysteinase [*Aeromonas hydrophila*]

>gi|169641123|gb|ACA61284.1| S-ribosylhomocysteinase [*Aeromonas hydrophila*]
MPLLDSFTVDHTRMAAPAVRVAKTMQTPNK
DTITVFDLRFCVPNQEILSERGIHTLEHLFA
GFMRDHLNGGVEIIDISPMGCRTGFYM
SLIGAPDEARVGAAWQAAMSDVLTVQEQ
GKIPELNEYQCGTYSMHSLEEAHAIA
RHVLERGIGVNRNDELALPEEKLKSL.

**Template >1JOE:A|PDBID|CHAIN|SEQUENCE:**
***HEAMOPHILUS INFLUENZAE***

MPLLDSFKVDHTKMNAPAVRI
AKTMLTPKGDNITVFDLRFCIPN
KEILSPKGIHTLEHLFAGFMRDHLNG
DSIEIIDISPMGCRTGFMSLIGTPNEQKVS
EAWLASMQDVLGVQDQASIPELNIYQCGSYTE
HSLEDAHEIAKNVIARGIGVNKNEDLSLDNSLLK

### Homology Modelling

The template protein in pdb format was processed for comparative modeling. The modeller latest version modeller9v2 that works in the command prompt with python basis, gives an

**Table 1.** Probabale Drugs Targeted for S-ribosylhomocysteinase a predicted by Ligprof

| Ligand | description | p-value | notes |
|---|---|---|---|
| KRI | (S)- 2- AMINO- 4- [(2S,3R)- 2,3,5- TRIHYDROXY- 4- OXO- PENTYL] MERCAPTO- BUTYRIC ACID | 0.019 | low significance |
| ZN | ZINC ION | 0.019 | low significance |
| RHC | 5- (3- AMINO- 4,4- DIHYROXY- BUTYLSULFANYLMETHYL)- TETRAHYDRO- FURAN- 2,3,4- TRIOL | 0.026 | low significance |
| HCS | 2- AMINO- 4- MERCAPTO- BUTYRIC ACID | 0.036 | low significance |
| ZN | ZINC ION | 0.180 | low significance |
| ZN | ZINC ION | 0.187 | low significance |
| ZN | ZINC ION | 0.306 | probably random occurrence |
| ZN | ZINC ION | 0.373 | probably random occurrence |
| HCS | 2- AMINO- 4- MERCAPTO- BUTYRIC ACID | 0.639 | probably random occurrence |
| SO4 | SULFATE ION | 0.919 | probably random occurrence common small ion |

**Table 2.** Comparison of Binding Motif for the Best Binding Ligand between the target S-ribosylhomocysteinase and the source

| Motif information (contacts) | | | | |
|---|---|---|---|---|
| Query: Position | Residue | HMM: Position | Sources | Residues |
| 21 | V | 21 | 1JQW_A | C |
| 35 | V | 35 | 1JQW_A | K |
| 88 | Y | 90 | 1JQW_A | Y |

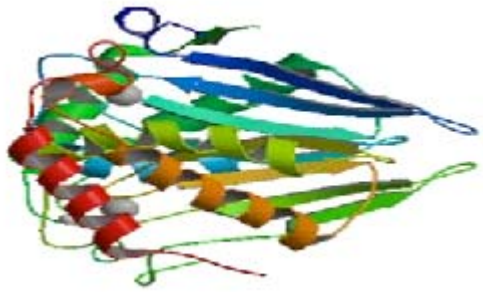

**Fig. 1.** 3D structure of the Template- 1JOE

accurate model and returns the dope score with which the best model has been predicted. In this project, of the 5 models predicted, the model with lowest energy and Dope score is taken as best model. Further, the best model has been verified with Procheck which shows the reliability of structure prediction. Through Ramachandran plot, it is clear that all the residues lie in the sterically allowed region. It revealed only minimum number of residues in the unfavourable state. The main chain bond angle plots as given by procheck

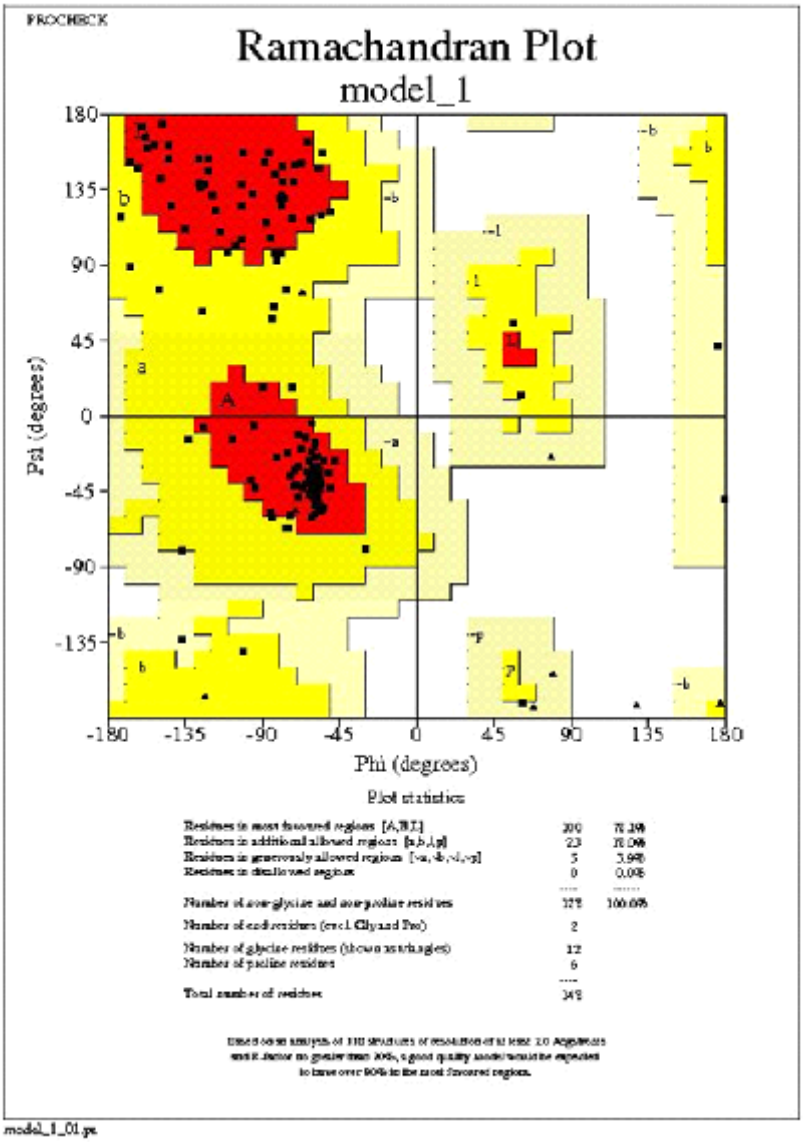


**Fig. 2.** Best Modelled Structure verified by Procheck Ramachandran Plot

shows only a minimal deviation as per Engh & Huber small-molecule data. The total free energy of the predicted structure was -6514.88 (Figure 2). Structure –Structure Alignment using DALI server: The pairwise comparison of protein structures were analysed by pairwise DaliLite server. Thus the alignment between the template and the best structure shows only minimum number of structurally non-equivalent residues that has the probability of forming loop region. The alignment between target and template provides the Z-score of 24.1 (Figure 3).

Three-state secondary structure definitions by DSSP (reduced to H=helix, E=sheet, L=coil) are shown above the amino acid sequence. Structurally equivalent residues are in uppercase, structurally non-equivalent residues (e.g. in loops) are in lowercase. Amino acid identities are marked by vertical bars.

**Identification of Probabale Drugs Targeted for S-ribosylhomocysteinase using LigProf server:**

LigProf is an attempt to transfer annotation about ligand neighbourhoods from known protein structures (contained in the PDB)

```
No:  Chain   Z    rmsd lali nres  %id PDB  Description
      1:  mol2-B 24.1  0.9  148   148   75 PDB

DSSP  LLHHHLLLLEEEEEEEEELLLLLEEEEEEEELLLLLLLLLLHHHHHHHHHHHHHHHHHLLL
Query VDHTKMNAPAVRIAKTMLTPKGDNITVFDLRFCIPNKEILSPKGIHTLEHLFAGFMRDHL   60
ident |||| | ||||| |||| ||  | |||||||||| || ||||  ||||||||||||||||
Sbjct VDHTRMAAPAVRVAKTMQTPNKDTITVFDLRFCVPNQEILSERGIHTLEHLFAGFMRDHL   60
DSSP  LLLLLLLLLEEEEEEEEELLLLLEEEEEEEELLLLLLLLLLHHHHHHHHHHHHHHHHHHL


DSSP  LLLLLEEEEEEELLLLLEEEEEEELLLLHHHHHLHHHHHHHHHLLLLLLLLLLLLLLLLL
Query NGDSIEIIDISPMGCRTGFYMSLIGTPNEQKVSEAWLASMQDVLGVQDQASIPELNIYQC  120
ident |||  ||||||||||||||||||| | |  |  || | | ||| || |  ||||| |||
Sbjct NGDGVEIIDISPMGCRTGFYMSLIGAPDEARVGAAWQAAMSDVLTVQEQGKIPELNEYQC  120
DSSP  LLLLLLEEEEEELLLLLEEEEEEELLLLHHHHHHHHHHHHHHHHHLLLHHHLLLLLLLL


DSSP  LLLLLLLHHHHHHHHHHHHHHLLEEELL
Query GSYTEHSLEDAHEIAKNVIARGIGVNKN  148
ident | |  |||| || ||  |  |||||| |
Sbjct GTYSMHSLEEAHAIARHVLERGIGVNRN  148

DSSP LLLLLLLHHHHHHHHHHHHHLLLEEEEL
```

**Fig. 3.** Select neighbours (check boxes) for viewing as multiple structural alignment or 3D superimposition. The list of neighbours is sorted by Z-score. Similarities with a Z-score lower than 2 are spurious. Each neighbour has links to pairwise structural alignment with the query structure, and to the PDB format coordinate file where the neighbour is superimposed onto the query structure

to protein domains of the Pfam database (Table 1). With the increasing amount of data provided by both high-throughput sequencing and structural genomics studies, there is a growing need for tools to augment functional predictions for protein sequences. Broad descriptions of function can be provided by establishing the presence of protein domains associated with a particular function. To extend the domain-based annotation, LigProf provides predictions of potential ligands that bind to a protein, as well as critical residues that stabilize ligands. A P-value statistic for estimating the significance of motif occurrence is provided for all sites. Since, HPS[2- AMINO- 4- MERCAPTO-BUTYRIC ACID] has the highly predicted 'P' value(0.639), which shows the high affinity of this ligand to bind with the predicted 3D structure of homocysteinase in it's predicted active site by the server itself. The comparison of contact position of the ligand with the predicted structure of S-ribosyl homocysteinase to that of the source present in the database is also provided (Table 2).

## CONCLUSION

Homology modelling/comparative modelling represents a starting point for studying structure function relationships which has many applications such as virtual screening. Hence, the target enzyme S-ribosyl homocysteinase which is

coded by LuxS gene can be well targeted for drug designing with its available 3D structure and analysis of ligand binding sites. Since, it is directly involved in both bio film formation and quorum sensing, the ability of insilico tools to predict the three dimensional protein structures give ways for drug targeting against *Aeromonas hydrophila*. The generated molecular models can be used further to study the structure function relationships. Thus the comfort, ability and accuracy of insilico tools in functional proteomics thereby indirectly involved efficiently in drug discovery.

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