LuxS quorum sensing system, its protein modeling and active-binding sites and phylogenetic analysis from *Aeromonas hydrophila*

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LuxS is commonly found in various bacterial species, like *A. hydrophila* which causes infection in fish, shrimps, and prawns and is a great threat to aquaculture industry as well as public health. It is an essential enzyme and highly conserved in various bacterial species, and has a wide range of functions such as involved in quorum sensing (QS), sporulation, virulence and synthesis of biofilm. This study focused on the prediction of 3D-sturcture of LuxS by template similarity and its ligand binding sites analysis to define its structure-function relationship. Primary structure analysis of LuxS examined that about 42% of residues content are alpha-helix, which makes it stable for three-dimensional structure homology. For the con struction of homology modeling of LuxS, crystal structure (5e68.1.A) has been used as a template and Swiss model as a work space. The validation of model by ProSA, SAVES, PROCHECK, PROSAII and RMSD. All results analysis shows that refined model is reliable and it has78.11% amino acids sequence similarity with the template,0.4Åas RMSD, and Z-score is -6.21 and Ramachandran plot analysis shows that 83.4% of residues found in the most favored regions where only 0.4% falls into the disallowed regions. Zinc ion ligand was predicted with highest MAMMOTH score and its binding residues His-54, His-58 and Cys-128 were analyzed by COACH-Meta server. LuxS phylogeny was constructed by sequences and structures of the most similar sequences were analyzed. *In silico,* the information has been generated in this work expects to be the first step towards the structure determination of LuxS in *A. hydrophila.*

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Abstract

LuxS is commonly found in various bacterial species, like *A. hydrophila* which causes infection in fish, shrimps, and prawns and is a great threat to aquaculture industry as well as public health. It is an essential enzyme and highly conserved in various bacterial species, and has a wide range of functions such as involved in quorum sensing (QS), sporulation, virulence and synthesis of biofilm. This study focused on the prediction of 3D-sturcture of LuxS by template similarity and its ligand binding sites analysis to define its structure-function relationship. Primary structure analysis of LuxS examined that about 42% of residues content are alpha-helix, which makes it stable for three-dimensional structure homology. For the construction of homology modeling of LuxS, crystal structure (5e68.1.A) has been used as a template and Swiss model as a work space. The validation of model by ProSA, SAVES, PROCHECK, PROSAII and RMSD. All results analysis shows that refined model is reliable and it has78.11% amino acids sequence similarity with the template,0.4Åas RMSD, and Z-score is -6.21 and Ramachandran plot analysis shows that 83.4% of residues found in the most favored regions where only 0.4% falls into the disallowed regions. Zinc ion ligand was predicted with highest MAMMOTH score and its binding residues His-54, His-58 and Cys-128 were analyzed by COACH-Meta server. LuxS phylogeny was constructed by sequences and structures of the most similar sequences were analyzed. *In silico,* the information has been generated in this work expects to be the first step towards the structure determination of LuxS in *A. hydrophila.*

KEYWORDS: *Aeromonas hydrophila*, LuxS, Quorum sensing, 3D-structure.

Introduction

The S-ribosylhomocysteinase (luxS) quorum-sensing (QS) system is found in various bacterial species**(Waters and Bassler et al., 2005)** and was primarily characterizing as the regulator of bioluminescence in *Vibrio harveyi* **(Surette, Miller and Bassler et al., 1999)**. The presence of luxS homologs in both Gram-positive and Gram-negative bacteria suggests that AI-2 is a universal language for interspecies communication **(Xavier and Bassler et al., 2003)**. Also LuxS is an integral component of the activated methyl cycle, which provides an alternative explanation for its widespread conservation **(Winzer and HARDIE K et al., 2003)**. The LuxS-encoding enzyme plays a role in the metabolism of S-adenosyl-methionine (SAM) and converts S-ribosyl-homocysteine (SRH) into homocysteine and 4,5-dihydroxy-2,3- pentanedione (4,5-DPD), and then reacts with water to form several different furanone **(Schauder, Shokat, Surette and Bassler et al., 2001, Sperandio, Torres, Jarvis, Nataro and Kaper et al., 2003, Winzer, Hardie and Williams et al., 2002)** whereas, 4,5-DPD autocatalytically hydrolysis and biosynthesis of autoinducer (AI) takes place**(De Kievit and Iglewski et al., 2000)** . The QS is broadly known due to the regulation of crucial mechanism like competence, sporulation, motility, biofilm formation, as well plays a important role in virulence. LuxS protein is also known S-ribosylhomocysteinase concerned to the synthesis of AI-2. According to SCOP system of protein classification, LuxS belongs to the super family of enzymes LuxS/MPP-like metallohydrolase. A notable characteristic of this protein is that it is one of the few enzymes able to cleave of thioether bonds without using a redox cofactor **(Pei and Zhu et al., 2004)**. In addition, several studies show that LuxS gene is highly conserved in various species like *A. hydrophila ,E.coli, V.cholera,* and *S.typhi,* but do not share any homology with another genes**(Rao, Pasha and Sowdhamini et al., 2016, Seshadri, Joseph, Chopra, Sha, Shaw, Graf, Haft, Wu, Ren and Rosovitz et al., 2006)**. The number of structural studies has been published on LuxS protein. First, obtained crystal structure of LuxSand showed

that, this protein was in homodimer, with six alpha-helices are covered with eight stranded beta-barrels, and the active site contains zinc ion, which is coordinated with highly conserved residues His-54, His-58, and Cys-126**(Ruzheinikov, Das, Sedelnikova, Hartley, Foster, Horsburgh, Cox, McCleod, Mekhalfia and Blackburn et al., 2001, Hilgers and Ludwig et al., 2001)**. Further, it was studied that, restriction of active site has been observed due to conformational changes in the protein, residues 125-131 involvement and residues around the N-terminus**(Hilgers and Ludwig et al., 2001)**.

In previous studies on evolution about LuxS protein showed that LuxS have evolved at the time divergence of prokaryotic phyla, which is based on large consent with a single subunit of ribosomal RNA tree of bacteria. According to genome-wide studies on LuxS gene in various bacterial genomes has shown that LuxS is widely found in bacterial domain whereas, AI-2 mediated signaling process function as a universal mode of interspecies communication **(Sun, Daniel, Wagner-Döbler and Zeng et al., 2004)**. On the other hand, some reports showed that, AI-2 examined as binding receptors, with authors suggestions, AI-2 mediated QS is restricted in some species like *vibrionales,* and gut pathogenic bacteria**(Rezzonico and Duffy et al., 2008)**.

However, by the dawn of large-scale bacterial genome sequencing projects and augmented recognition of the role of LuxS in growth and virulence of different bacterial pathogens, at the wide range of perspective is needed to know the structure and function of the LuxS protein from *A. hydrophila*. Therefore, this study aims to examine the 3D-structure of LuxS protein model to recognize the active sites and its ligand binding sites and Phylogenetic analysis based on sequence and structure.

Methodology

Identification of LuxS

The LuxS amino acid sequence was retrieved from Uniport database (A0kG57). Subsequent to that, from the transcript the full length open reading frame (ORF) chosen as putative coding sequence and resultantly protein determined as s-ribosylhomocysteine lyase. Furthermore, protein sequence was submitted to the InterPro protein families' database and Evolutionary classification of protein domains (ECOD) database.

Primary, secondary structure and functional site prediction analysis

The primary structure analysis was done due to get the insights and made the estimation of hydrophobic and hydrophilic residues. For the computation of physicochemical parameters, Expasy's PortParam was used. Furthermore, for the prediction of secondary structure, its class identification and α-helical, β- strand and the coiled region percentage was calculated by SOPMA Expasy tool. Inter-pro protein sequence analysis and classification were used to find out the functional regions present LuxS.

Sequence alignment

The target FASTA sequence of LuxS was used as a query sequence in PDB to find the comparative modeling **(Berman, Westbrook, Feng, Gilliland, Bhat, Weissig, Shindyalov and Bourne et al., 2000)**. Commonly this search is made due to the comparison of the target sequence with the sequence of every sequence in the database. Blast (Basic Local Alignment Search Tool) has done to compare the target sequence with the similar sequence **(Altschul, Madden, Schäffer, Zhang, Zhang, Miller and Lipman et al., 1997)** against Protein Databank. The BLAST results give up X-ray structure of 5e68.1A chain a (template) with an Expect value (E value) of 4.9e-118 and 78.11% similarity to the target protein (**Table S3**). The Swiss-PDB viewer was used to construct a structure based alignment and SWISS-MODEL was exercised in the optimized mode to minimize energy.

Protein 3D-structure modeling and superposition

LuxS (169 amino acids) protein sequence was used as the query sequence for the comparative homology modeling on the basis of template 5e68.1A by using Swiss- model web Server **(Arnold, Bordoli, Kopp and Schwede et al., 2006)**. Structure assessment and quality check was carried out at Swiss-model server using Procheck **(Kopp and Schwede et al., 2006) (Laskowski, MacArthur, Moss and Thornton et al., 1993)**. The best model selection was done by PROCHECK on the basis of Stereochemistry. The superposition of both model and template was generated by using superpose web server **(Maiti, Van Domselaar, Zhang and Wishart et al., 2004)** and viewed by UCF-Chimera and RR Distance maps was created to measure the pair wise distance of residues between both superimposed chains**(Chen, Huang and Ferrin et al., 2014)**.

Validation of model ProSA

ProSA program is a tool extensively applied to verify 3D models of protein structures for feasible errors. SAVES is a tool applied for Errat value prediction; validate 3d plot and Ramachandran plot determination. ERRAT is a protein structure confirmation algorithm that is particularly well-matched for the evaluation of refinement and model building perfection. The program working by examines the statistics of non-bonded interactions of different atom types. Procheck A multipurpose protein structure analysis program **(Laskowski, MacArthur and Thornton et al., 1998)** was used for the confirmation of protein structure and models by verifying the parameters such as quality of Ramachandran plot, planarity of peptide bonds , interaction of bonds, hydrogen in main chain, bond energies and various standard deviations for the refinement of structures**(Morris, MacArthur, Hutchinson and Thornton et al., 1992).**

RMSD

Root Mean Squared Deviation (RMSD) is usually exercised to signify the distance between two objects. In a structural sense, this value specifies the degree to which two three-dimensional structures are parallel. The structure similarity depends on RMSD

values, such as if lower the RMSD value, structures are more similar and vice versa. The RMSD value between the template and the model structure was calculated by means of UCF-chimera.

Ligand and binding sites evaluation

Ligand and binding sites were predicted by 3D ligand site- ligand binding site prediction server (http://www.sbg.bio.ic.ac.uk). For further verification, COACH-server was used to study the ligand and its binding positions (http://zhanglab.ccmb.med.umich.edu/COACH).

Construction of phylogeny based on sequence and structure

LuxS sequences were collated from uniport protein database. The multiple sequence alignment (MSA) was carried out by using ClustalW **(Thompson, Gibson and Higgins et al., 2002)** and MEGA 7.0 **(Kumar, Stecher and Tamura et al., 2016)** was used to generate the Phylogenetic tree and using a neighboring-joining method with Poisson distribution, pair-wise distance and bootstrap values of 1000 replications**(Archak and Nagaraju et al., 2014)**. For the 3-D structure –based Phylogenetic analysis of structural comparison, one representative of each clade was taken from sequence-based neighbor-joining tree. The prediction of the selected structure of each protein was modeled by homology modeling (*Cronobacter malonacticus, cronobacter muytjensii, Salmonella typhi,Ewrinia sp, Aeromonas hydrophila,Hafnia paralvei, Pragia fontium,Hemophillus influenza, Helicobacter pylori,Streptococcus suis*).The structural alignment of modeled structures and generation of phylogeny has been carried out via multiseq **(Roberts, Eargle, Wright and Luthey-Schulten et al., 2006)**.

Results

Synthesis of AI-2 by LuxS system

Most of the bacterial species, including *A.hydrophila*, have conserved luxS homologs, which is responsible for the synthesis of LuxS enzyme. The conversion of S-adenosylhomocysteine by Pfs enzyme into substrate S-ribosylhomocysteine and adenine then substrate cleaved via LuxS into 4,5-dihydroxy-2,3-pentanedione (DPD) and homocysteine, which is the precursor of AI-2 **(Schauder and Bassler et al., 2001).** When DPD reacts with water molecule it goes into cyclization for the formation of AI-2 **(Cook and Federle et al., 2014)** (**Figure1**). In LuxS containing bacteria at high cell density AI-2 plays a potential role for the intercommunication among bacterial species **(Xavier and Bassler et al., 2003, Plummer et al., 2012)**. At large scale LuxS system has been studied well in gram-negative bacteria, like *A.hydrophila* and marine *γ-proteobacterium Vibrio harveyi* **(Schauder and Bassler et al., 2001)**. LuxP is well studied into *V.harveyi* , responds to AI-2 **(Chen, Schauder, Potier, Van Dorsselaer, Pelczer, Bassler and Hughson et al., 2002)** and the bacteria have not LuxP also responds to AI-2. Moreover, other receptors also found which are responsible for the biosynthesis of AI-2 precursors, such as, LsrB (*Bacillus, E.coli* and *Salmonella enterica*) and rbsB in *H. influenza* (reviewed in**(Pereira, Thompson and Xavier et al., 2013)**. However, in most of gram-negative bacteriaAI-2 receptor gene LuxP is well defined although in gram-positive bacteria till today need to studies at extensive levels.

In both G-positive and G-negative bacterial species including *A. hydrophila*, LuxS enzyme is playing crucial role in the regulation of transcription of various genes like those connected with transport of nucleotides, metabolism, synthesis of cell wall or membrane**(Shao, Shang, Yang, Sun, Li, Guo, Wang, Zou, Wang and Lei et al., 2012)**. While, the deregulation mechanism of those genes found in these pathways is still ambiguous. Furthermore, LuxS system of *A. hydrophila* performs a vital role in the *in vitro* biofilm synthesis. It has been studied that the mutant strain of LuxS remained to fail to produce mature biofilm**(Lynch, Swift, Kirke, Keevil, Dodd and Williams et al., 2002)** in addition to

that, increased concentration of salinity and enhanced incubation period reduced biofilm formation, motility, protease biogenesis and quorum sensing **(Jahid, Mizan, Ha and Ha et al., 2015)**. Mutation into AI-2 synthase gene LuxS reduces virulence towards host when AI-2 added from exogenous sources it becomes virulent **(Defoirdt, Bossier, Sorgeloos and Verstraete et al., 2005)**.

Architecture of LuxS

The gene length consists of 507 nucleotide base pairs and coded for 169 amino acids long protein. This protein contains one domain 4-162 amino acids named as metalloenzyme, LuxS/M16 peptidase-like. Metalloenzyme domains are found in two–layer alpha/beta structure luxS (S-ribosylhomocysteine (EC: 4.4.1.21) while metallopeptidases have close vicinity M16 family. These domains have an identical motif active site HxxEH found in the core helix however differ in one of the metal-binding residues. Whereas, LuxS is an iron-dependent homodimer metalloenzyme contains two tetrahedral metal-binding sites same as peptidases and amidases , also it has an extra N-terminal strand**(Hilgers and Ludwig et al., 2001, Rajan, Zhu, Hu, Pei and Bell et al., 2005)** (**Figure 2**).

Primary, secondary structure and domain analysis

The number of amino acids, molecular weight, and all the charged residues with positive and negative charges, grand average of hydropathicity (GRAVY), theoretical isoelctric point (pI) and each amino acids composition is shown in **(S1Table)**. The results demonstrate that the LuxS seems stable **(Guruprasad, Reddy and Pandit et al., 1990).** The target protein is good for 3D modeling as the alpha helix content is 41.42 % which will make the protein stable **(FigureS1 &S2).**The functional domain **(S2Table)** has been found in LuxS as metalloenzyme belongs to the family of S-ribosyl homocysteinase.

Modeling of LuxS

The tertiary structure of the protein was built from its secondary structure element which is responsible for the formation of distinct domains. The 3D-structure of *LuxS* model has predicted by comparative modeling via Swiss workspace based on target sequence alignment to the template. As a result, hypothetical protein model was generated and saved as PDB output file. The 3D structure of model and template is shown in (**Figure 3).** The alignment between 5e68.1A template and target is shown in **(Table 3S).**

Evaluation, validation, and superposition of template and model

The output result file of ProSA program shows the scores and energy plots which give information about possible errors highlighted in spots into the protein structure **(S3 Figure**). For the verification of the accuracy of protein generated model was verified by PROCHECK validity report. All of the parameters comparison were justified with those proteins have well-refined structure and same resolution. The most important chain parameters designed into Ramachandran plot quality, peptide bond planarity, bad non-bonded contacts, bond energy of hydrogen chain, alpha-carbon chirality and other G parameters. In the Ramachandran plot analysis, the residues were classified according to their regions in the quadrangle. The Ramachandran map for LuxS is shown in **(S4 Figure).** Backbone superposition is performed to know the differences and identities between the 3D structure of template 5e68.1A and predicted model. It is shown in **(Figure 4 A),** the topologies of both structures are similar, but some minor differences can be noticed between them. Further, verification of superimposed structures RR distance map was generated by UCF-Chimera tools. In combined map of two chains residue-residue contact or non contact differences is computed such as cutoff distances. The average distances and standard deviations of the corresponding pair of residues is depicted in different colors. Those pairs do not bring a change into two conformations (close together) depicted with white color to gray(far apart) whereas those pair of residues whose distances are at short average distance shown in red color to blue (long

average distance) (**Figure 4 B**). It ensures that predicted LuxS model is valid for further structure based studies. The RMSD (Root Mean Square Deviation) between predicted model and template is 0.4 Å.

Ligand, active binding sites, and topological features analysis

Zinc (Zn) ion ligand was predicted by highest MAMMOTH scores. Zn ion binds with four amino acids HIS 54, 58, CYS 128 and HIS 134 **(Figure 5A.**).While Zinc same ligand binds with HIS-54, 58 and Cys-128 was predicted by COACH-meta server with a high score (**Figure5B**). The active center is formed by the presence of conserved residues in LuxS, based on our sequence alignment analysis, near about 30 residues are found in a conserved region **(Figure 6B).** The metal cofactor is located in the center of the conserved cluster **(Figure 6A)**, which support our identification of active site. The initial identification of metal was predicted by COACH-meta server as mentioned above. The residues His-54, 58 and Cys-128 are coordinated with metal by single monomer chains. In the central region of α1 His-54 and 58 is found which coordinate with metal via N-termini atoms, whereas, cys-128 is found in the extended loop. These residues and water molecules complete the formation of the coordinated sphere, and zinc binding takes place with a tetrahedral geometry of residues. The important interatomic distances were found as His 58 NE -Zn, 1. 902 Å, His 54 –NE Zn 2.821 Å, Cys 128 Sg-Zn 2.611 Å and O (HOH)-Zn 1.82 Å **(Figure 6A).** From the geometry and the presence of solvent ligand assumed that this metal molecule plays more catalytic rather than structural role. Furthermore, those sequences and motifs are similar with metal binding sites also observed their conserved sequences to peptidases and amidases, instead of that LuxS might have a function in the biosynthesis of autoinducer-2(AI-2) like hydrolase **(Hilgers and Ludwig et al., 2001)**. However, by these results, we can disturb interatomic molecular interactions chemically and may be biosynthesis AI-2 is blocked and quorum sensing will prevent.

Molecular phylogeny based on LuxS protein sequence

In a phylogenic analysis of LuxS proteins sequences about 20 bacterial species were used by the neighbor-joining tree, species are together in one clade have a similar family (**Figure 7**). In all LuxS orthologs, hist-54-his-58-cys-128 metal ion (Zn) binding triad and an active site turn-causing motif of HxxEH were conserved among other LuxS orthologs. As per routine measure of structural similarity between two homologous protein structures is the RMSD of topologically corresponding C-alpha atoms and then used for Phylogenetic analysis. The structure based Phylogenetic tree (**Figure 8)** was constructed by using RMSD value (0.10 Å) of structural alignment, that was different from the Phylogenetic tree was generated based on sequence alignment. *A. hydrophila* has a distinct cluster with *cronobacter* species whereas it grouped together with *H.influenzae, S.suis, and H.pylori*.

Discussion

The LuxS has an essential contribution in quorum sensing even at high cell density produces AI-2 signaling molecules for the intra-species communication**(Xavier and Bassler et al., 2003, Plummer et al., 2012)**.The comparative homology modeling of proteins is done based on the templates to predict of 3D-structure of the target sequence. Homology modeling is merely the modeling method that can provide models with a root mean square error > 2Å. For the reliable homology modeling have a basic requirement to find the similarity between target and template. That's why we have searched and found high sequence identity between target and template, which was better to use a crystallographic structure of 5e68.1A as a template for structure prediction of target sequence via homology modeling. In order to deal with predicted models, divided into three categories such as low accurate models have target- template alignment identity less than 30% therefore low accuracy models should be treated with great care. While

accurate medium models are those model obtained via target-template sequence similarity between 30-50% and must be 85% of their C-alpha atoms within 3.5 Å at the right position. This type of models is used at the wide range of biological research applications, like to examine of ligand binding position via the designing of site-directed mutants with altered binding efficacy and computational screening of small potential molecules or inhibitors from the variety of databases. The third category of model accuracy depends on the protein sequence similarity should be more than 50%, then the structure has predicted from that sequence is capable of comparing with that structure have 3 Å resolutions, and this is considered for ligand docking and drug design computation. If the sequence similarity is more than 90%, it is useful for the description of the active site**(Marsden and Orengo et al., 2008).**

Further, best-predicted model was analyzed by PROCHECK **(Baker and Sali et al., 2001)**. Ramachandran plot shows that about 83.4% amino acid residues were found in the most favored region, while 11.4% was in allowed region and 4.8% residues in outlier region and 0.4% in disallowed region. Overall, the score was very near to 100% which means the model has a suitable stereo-chemical quality**(Kelm, Shi and Deane et al., 2010).** Low RMSD analysis between target and template it is responsible for strong homology (in other words lower RMSD, structure similarity is more and vice versa). The Z-score specifies model quality, total energy deviation measurement according to the distribution of energy from random conformations **(Kelm, Shi and Deane et al., 2010) .**The Z-score analysis was done due to knowing the score of targeted protein is whether found in the range of similar proteins, which are belonging to same groups. The z-score value can be seen in (**S3 Figure**) of the generated model (-6.21) and is positioned in the region of x-ray determined proteins and this value found very close to the template (-6.83). Due to the closeness of z-scores means targeted model is considered reliable for further studies. The substantiation between template and model was done by superimposition

of both chains and the average distances and standard deviation between pair of residues was computed and a combine RR distance maps has been generated. The prediction of ligand and its binding was predicted by COACH-meta server. In LuxS three ligand binding site namely His-54, 58 and Cys-128 and interatomic interactions have been studied. In addition to that, we have done its Phylogenetic analysis on the basis of sequence alignment with homologs conserved species contains a similar sequence of LuxS and then phylogeny was also studied on the basis of structures similarity. *A. hydrophila* has a distinctive cluster with *cronobacter* species, while it grouped concurrently with *H.influenzae,S.suis, and H.pylori*.

Conclusion

Summing up, it is explicit from LuxS, produces AI-2, which is responsible for quorum sensing signals for the intercommunication of *A. hyhrophila* ATCC7966. Based on the template structure it is evidently observed that the theoretical structure generated is structurally similar to the template structure and development of ligand and its binding sites has been found of the LuxS enzyme. On the basis of our findings, experimental work is needed to studies deep insights at structure level.

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Figure 1

Synthesis of AI-2 by QS LuxS system in *A. hydrophila*

The LuxS homologous gene is labeled in this diagram as LuxX,Y& Z. The Psf enzyme converts S-adenosylhomocysteine (SAH) into S-ribosylhomocysteine (SRH) and adenine. LuxS breaks SRH into AI-derivatives/precursors like DPD and homocysteine, and then DPD reacts with a water molecule and goes cyclization for the synthesis of AI-2. After synthesis AI-2 molecules come out of the bacterial cell via membrane proteins and play their role in quorum sensing.

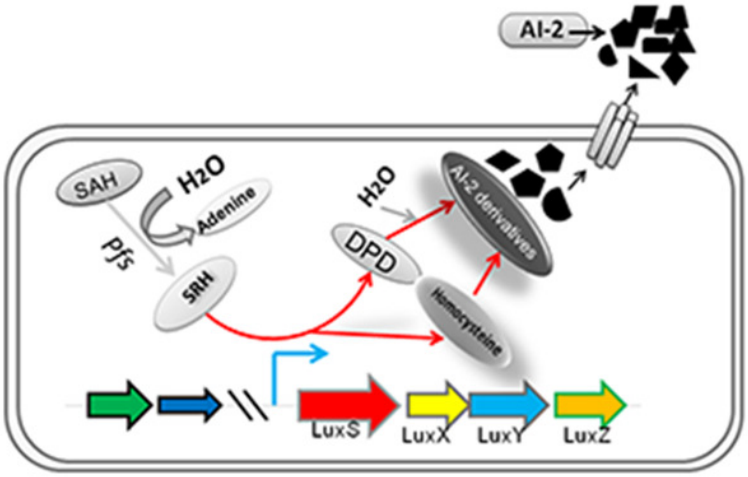

Figure 2

Architecture of LuxS gene.

This gene (green 507bp) after the process of transcription and translation is expressed into metalloenzyme, LuxS/M16 peptidase like functional domain (4-162) from N-C terminal depicted at the bottom in (green) with InterPro protein families database ID.IPR011249.

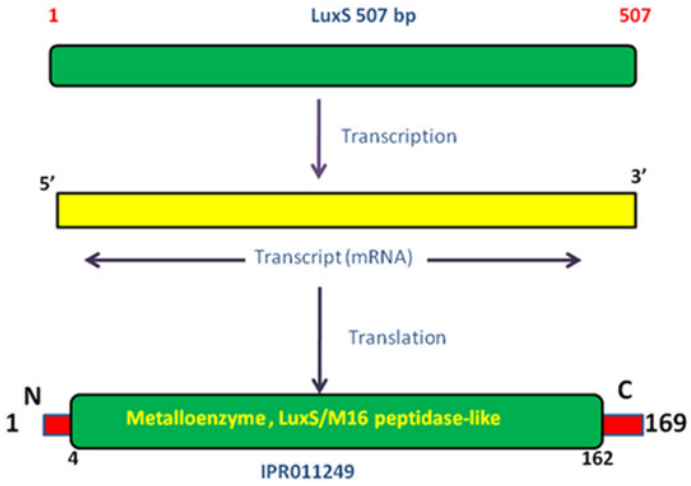

Figure 3

LuxS protein model prediction on the basis of template.

(A) 3D structure of the template (5e68A) obtained from PDB; **(B)** 3D structure of *LuxS* predicted using 5e68A as the template. Red (alpha helices), yellow (sheets) and green (loops).The difference between template and predicted model is found at the C-terminus. The template contains one extra beta sheet shown in black circle (about two residues long) and helices are also longer, whereas in predicted model at C-terminus helices are shorter, longer loops and last on residue found in the loop.

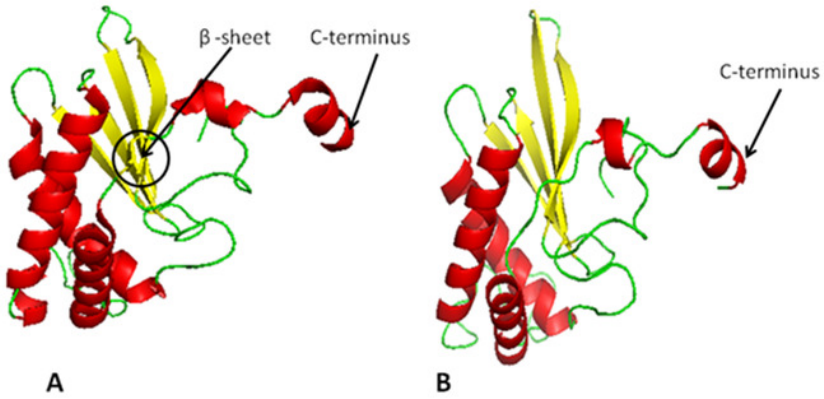

Figure 4

Superposition of LuxS model with template and RR Distance map generation.

(A) Superposition of predicted LuxS model (red orange color) and template 5e68A (hot pink color). **(B)** RR Distance Maps creates a color-coded combined map of superimposed two chains of LuxS predicted model and template. In a combined map, the average (intrachain) distances and standard deviations are shown. Both quantities can be shown on the same map with different dimensions of color.

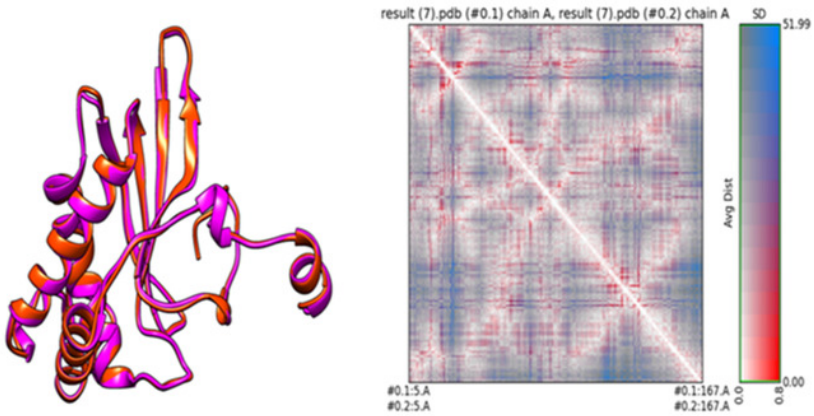

Figure 5

Ligand binding sites prediction analysis.

(A): Zinc-ligand (sphere), predicted by MAMMOTH highest score and binding sites are labeled with amino acid numbers. **(B)** Zinc-ligand (red) predictions by using of COACH sever and labeling with amino acid numbers.

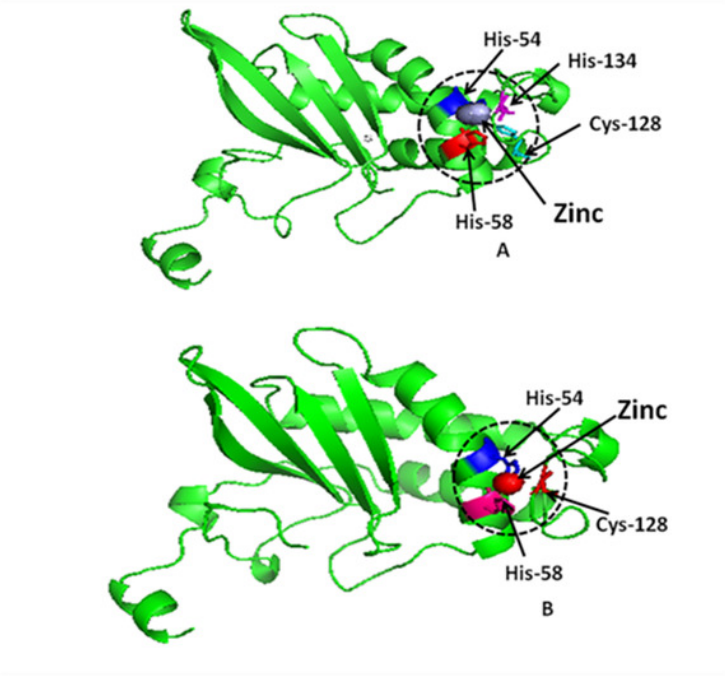

Figure 6

Stereo view of Zn-ligand cluster and the interatomic distances of the active site, and sequence alignment of LuxS.

(A): Monomer topological structure of LuxS shows the features of helices, sheets and the Zinc-binding sites (*top left*). Helices and sheets are labeled with α1-4 and β1-4 respectively. Zinc is depicted into a black sphere and the protein ligands, His-54, 58 and Cys128 are represented with sticks and ring shapes. **(B)** Sequence alignment of LuxS from selected organisms and conserved residues among sequences are highlighted in red. Zn ligand binding residues are shown in the red heart while cysteine is oxidized denoted in the black star.

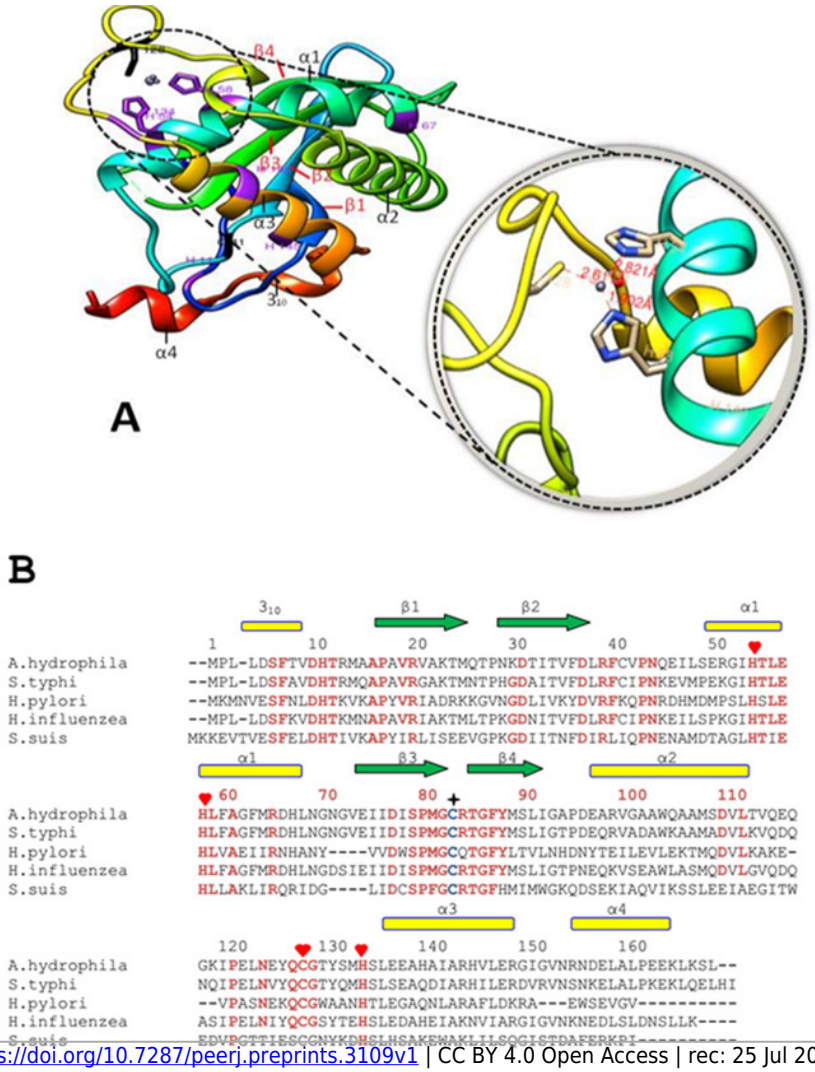

Figure 7

Phylogenetic analysis of LuxS.

Phylogenetic analysis of LuxS protein sequences shows 20 species grouped with respect to family. In parenthesis Uniport ID's are given in braces while bootstrap values are presented on the neighbor joining tree (red).

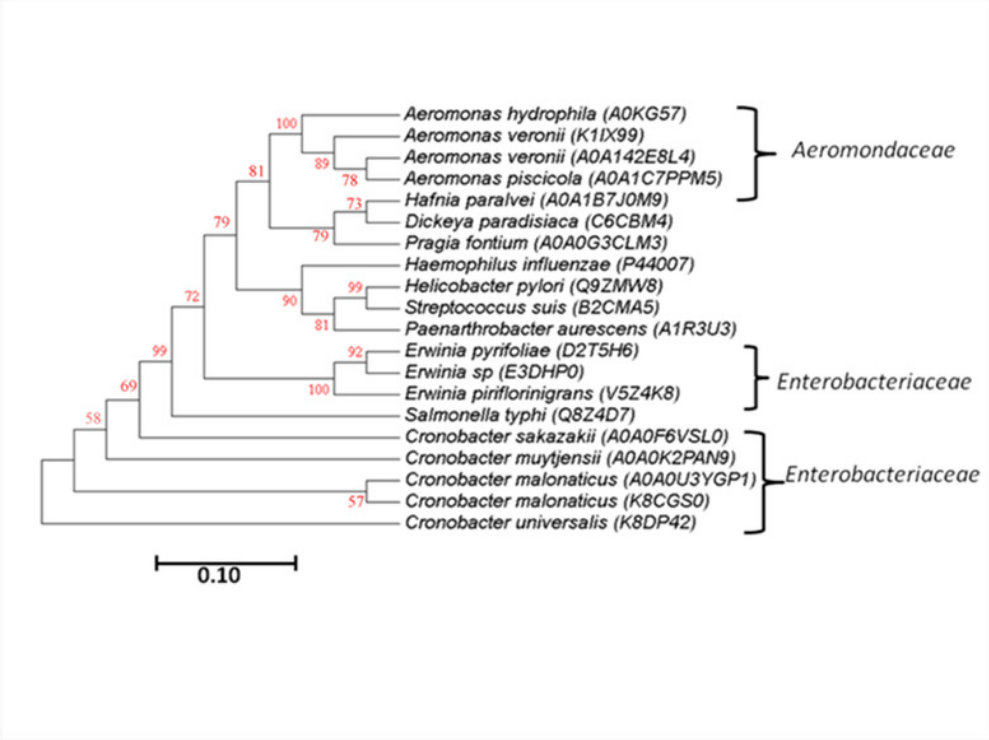

Figure 8

Phylogenetic tree of LuxS structures

Phylogenetic tree of LuxS structures of 10 bacterial species constructed based on root mean square deviation values. The structures, shown against species were modeled using crystal structure of (5e68). *A. hydrophila* has a distinct cluster with *cronobacter* species, whereas it grouped together with *H.influenzae,S.suis and H.pylori*.