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INSILICO QUORUM SENSING ANALYSIS OF LuxS PROTEIN AND TO STUDY ITS PHYLOGENY IN SELECTED BACTERIAL SPECIES

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ABSTRACT: Quorum sensing (QS) is an urbane cell-to-cell signalling mechanism that allows bacteria to share information about cell density and adjust their gene expression accordingly. LuxS plays a critical role in the biosynthesis of the autoinducer-2 (AI-2), which performs wide-ranging functions in bacterial communication, and especially in quorum sensing (QS). The AI-1 and AI-2 autoinducer systems are among the best characterized bacterial QS systems at the genetic level. In this study, we have done *in silico* QS analysis of LUXS protein and studied its phylogeny in selected bacterial species. The findings highlight that LuxS is a conserved yet adaptable protein crucial for quorum sensing through AI-2 synthesis. While core functional motifs are preserved, sequence variations highlight evolutionary divergence among bacterial species. These findings enhance our understanding of LuxS protein evolution and its role in bacterial communication.

Keywords: Autoinducer 1(AI 1), Quorum sensing, cell to cell communication, LuxS protein,

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1. INTRODUCTION

Bacteria have developed advanced forms of communication for the coordination of group activities in response to changes in cell population density. This is known as Quorum Sensing (QS), and it makes use of the production, secretion, and detection of small signaling molecules called

autoinducers (AI) [1]. The communication is based on accumulation of AI molecules, which is detected outside of the cells as the density of bacteria increases [2]. The autoinducers will bind to specific receptors known as 'sensing' proteins when the concentration reaches the critical threshold. The binding triggers a cascade of signal transduction pathways controlling a multitude of physiological processes like the forming of a biofilm, regulating the expression of virulence factors, sporulation, developing resistance to antibiotics, and even undergoing competence for genetic transformation [3,4,5]. Different strategies are used within Gram-positive and Gram-negative bacteria for QS. Different signalling and receptor molecules are used on them. Gram-negative bacteria primarily rely on N-acyl homoserine lactones (AHLs) for QS, whereas Gram-positive bacteria depend on oligopeptides that bind to sensor kinase systems [6,7,8]. AI 2 is notable because it is considered a universal signalling molecule since it can be found in over 55 species of bacteria, making it one of the most fascinating pathways. Unlike AHLs and oligopeptides, AI-2 is not species-specific and allows interspecies communication, particularly in complex microbial communities such as those found in aquatic environments, the human gut microbiome, and polymicrobial infections. In the functioning of AI-2 signaling, the LuxS protein is of great importance, being a key enzyme for the biosynthetic pathway of AI-2. LuxS's activity involves hydrolysis of S-ribosylhomocysteine (SRH) to yield 4,5-dihydroxy-2,3-pentanedione (DPD), which spontaneously cyclizes into AI-2 [9, 10,]. In addition to its functions in QS, LuxS is also part of activated methyl cycle (AMC) in which S-adenosylmethionine (SAM) is an active methyl group donor in the cellular metabolism and is regenerated with the help of LuxS [11]. Remarkably, LuxS is a member of the LuxS/MPP-like metallohydrolase superfamily and is among the very few known enzymes that can cleave thioether bonds without a redox cofactor incorporating into the processes. Research from comparative genomics indicates that the LuxS gene seems to be ubiquitous among bacterial species such as *Escherichia coli, Vibrio cholerae* and *Salmonella typhi* [12,.13,14]. Its conservation is, however, paradoxical, because the LuxS protein is not homologous to other QS genes, disclosing its independently unique evolutionary divergence. Analyzing evolutionary conservation together with the structural and functional diversification of LuxS remains important, mainly regarding pathogenic organisms from waterborne infections where AI-2 dependent QS is associated with infection and persistence in the environment [15]. In view of these important aspects of LuxS, the present study was conducted to analyze the sequence similarity to identify and compare LuxS protein sequences across different bacterial species.To check the conserved motif identification by detecting functionally important motifs and active site residues in LuxS. The Phylogenetic Analysis was done to explore the evolutionary relationship of LuxS among selected bacterial species. This study aims to enhance our understanding of LuxS-mediated quorum sensing and its role in bacterial communication networks. These insights could have broader implications in fields such as microbial ecology, infectious disease control, and antimicrobial drug development.

2. MATERIALS AND METHODS

Retrieval of LuxS sequence: was retrieved from Uniprot (www.uniprot.org) [16].

BLASTP: The similar protein sequences to the query were retrieved using BLASTP. (https://blast.ncbi.nlm.nih.gov)[17]. Protein sequences of LuxS from were selected from*Bacillus subtilis, Escherichia coli Deinococcus radiodurans, Streptococcus pyogenes, Salmonella paratyphi, Cutibacterium acnes, Campylobacter jejuni, Vibrio campbellii* the BLAST P results.

Clustal Omega: Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) [18] was used for multiple sequence alignment of LUXS from all the selected bacterial species.

Phylogenetic analysis: Phylogenetic tree generated by Clustal omega was analysed to see the evolutionary relationship between the LuxS protein sequences from the selected bacterial species.

Pfam analysis for Domain identification: The identification of the protein family in LuxS protein sequences was done using Pfam (Protein family) database (https://www.ebi.ac.uk/interpro/pfam/)[19].

3. RESULTS AND DISCUSSION

The multiple sequence alignment (MSA) of the LuxS protein across various bacterial species, generated using CLUSTAL Omega, provides insights into the evolutionary conservation and functional importance of specific amino acid residues (**Figure 1**). Highly conserved regions often correspond to active sites, binding interfaces, or structural motifs essential for the protein's function

Figure 1: Multiple sequence alignment of the LuxS protein from different bacteria done using CLUSTAL Omega showing conservation of protein.

In LuxS, these conserved residues may be involved in its role in the activated methyl cycle and quorum sensing pathways. The pattern of conservation and variability can shed light on evolutionary

pressures. Highly conserved residues suggest strong purifying selection, maintaining essential functions across species. In contrast, variable regions might be under diversifying selection, allowing adaptation to different environmental niches or regulatory mechanisms.

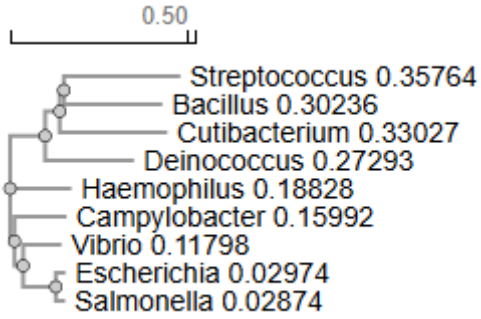

Figure 2: Phylogram showing the evolutionary relationships of LuxS form different bacteria

This phylogenetic tree (phylogram) illustrates the evolutionary relationships between the LuxS proteins from various bacterial species (**Figure 2**). The branch lengths are proportional to the genetic distance, providing insights into evolutionary divergence. The LuxS protein shows varying degrees of conservation and divergence across bacterial species. Organisms with longer branch lengths may have undergone more mutations, possibly adapting LuxS to specific ecological niches or regulatory needs. The close grouping of *Escherichia coli* and *Salmonella* aligns with their known phylogenetic relationship as closely related Gram-negative bacteria [20]. This phylogram highlights evolutionary trends in the LuxS protein, with conserved sequences among related species and distinct divergence patterns in others like *Streptococcus* [21,22] Such insights can be valuable for understanding functional differences, evolutionary pressures, and potential targets for bacterial control strategies

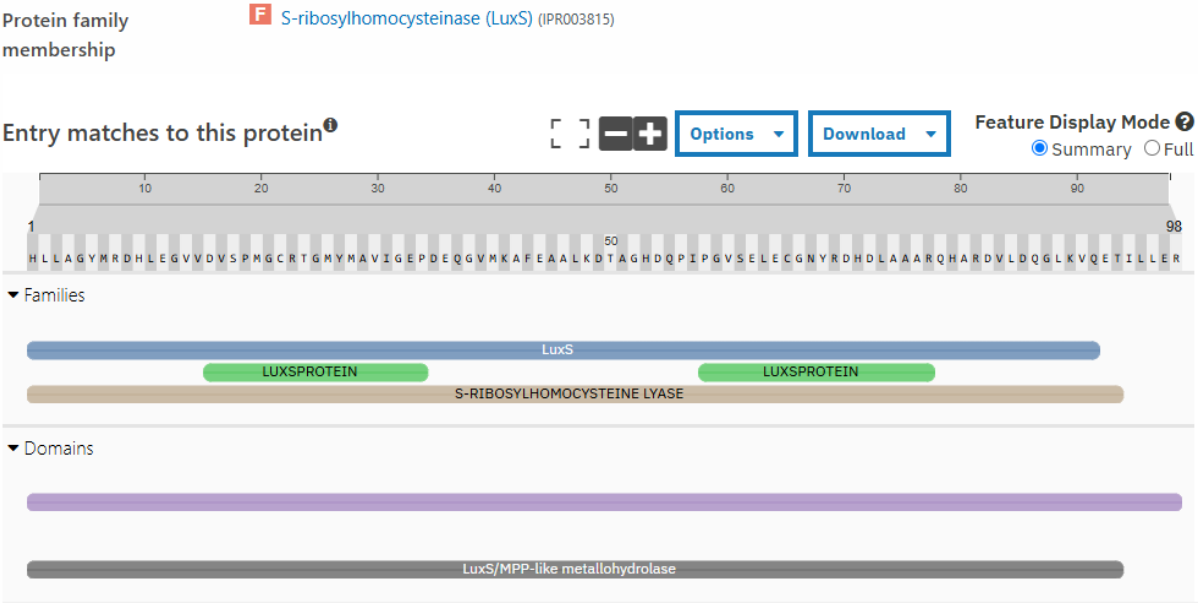

Figure 3: Interpro results shows the LuxS protein from all the bacteria belong to S ribosylhomocysteinase family. This is a representative picture for *Deinococcus radiodurans*

The InterPro result sheds further light on the protein LuxS from the organism *Deinococcus*

radiodurans confirming its assignment and domain architecture (**Figure 3**). The LuxS protein is described to belong to the S-ribosylhomocysteinase (LuxS) family (IPR003815). This family is of great importance in the activated methyl cycle, as well as in quorum sensing where they produce an autoinducer-2 (AI-2) signaling molecule and the results were in agreement with Houdt et al. (23). LuxS protein family regions (green bars) are depicted in the diagram as the most distinctive features of LuxS enzymes. The S-ribosylhomocysteinelyase region (brown bar) marks the part of the proteincatalytic domain that degrades S-ribosylhomocysteine to homocysteine and AI-2. The LuxS/MPP-like metallohydrolase domain (grey bar) is important for the catalytic function of the enzyme, especially the mechanisms that involve direct cleavage of peptide bonds involving metal ions. The violet bar shows representation of wider domain of the metallohydrolase superfamily domains which confirms the position of LuxS as a metalloenzyme. It is one of the metalloenzymes that catalyze the cleavage of thioether bonds in the absence of redox cofactor [24] The presence of these conserved regions suggests that the LuxS protein in *Deinococcus radiodurans* shares key structural and catalytic features with LuxS homologs from other bacteria. The metallohydrolase domain is particularly vital for enzymatic activity, indicating a potential target site for inhibitors aimed at quorum sensing disruption[25].. Since LuxS is central to AI-2 production, this alignment supports its conserved function across diverse bacterial species. The presence of conserved domains across bacterial species aligns with LuxS's fundamental role in microbial communication and metabolism. Variations may indicate species-specific adaptations or regulatory mechanisms. The multiple sequence alignment reveals highly conserved residues, especially in regions critical for LuxS's enzymatic activity (e.g., catalytic sites and metal-binding residues). *Escherichia* and *Salmonella* show minimal divergence, clustering closely in the phylogenetic tree, which aligns with their shared evolutionary background within the Enterobacteriaceae family. *Streptococcus* and *Cutibacterium* show more significant divergence, suggesting functional or structural adaptations in their LuxS proteins. The greater branch length for *Streptococcus*(0.35764) in the phylogenetic tree indicates a higher rate of evolutionary change in its LuxS sequence, potentially reflecting unique adaptations. Despite sequence variation, all species maintain core conserved motifs related to the LuxS/MPP-like metallohydrolase domain, confirming that LuxS retains its fundamental enzymatic role in the production of autoinducer-2 (AI-2) across diverse bacteria [26].The presence of the S-ribosylhomocysteinelyase domain in all species indicates a preserved mechanism for generating AI-2, reinforcing the essential role of LuxS in bacterial quorum sensing and metabolic processes. The observed sequence variations may point to Species-specific regulation of AI-2 production and Adaptations to different ecological niches or bacterial lifestyles (e.g., commensal, pathogenic, or environmental bacteria). The conserved metallohydrolase domain suggests LuxS's catalytic mechanism is crucial for its function, while variable regions may relate to structural flexibility or regulatory differences [27]. The conservation of key functional domains makes LuxS an attractive

target for designing LuxS inhibitors or AI-2 blocking agents to disrupt quorum sensing in pathogenic bacteria. Species with more conserved sequences (e.g., *Escherichia* and *Salmonella*) may be more vulnerable to such inhibitors, whereas highly divergent sequences (e.g., *Streptococcus*) may require tailored strategies.

4. CONCLUSION

Taken together, LuxS produces AI-2, which is responsible for quorum sensing signalling in the intercommunication among bacterial species. *In silico* threading methods were used to predict the LuxS protein structure, which revealed a high degree of sequence conservation, structural similarity and functional relevance in AI-2 mediated quorum sensing. Phylogentic analysis indicates evolutionary divergence while maintaining core catalytic features of LuxS. These results provide a framework to further develop a complete understanding of LuxS and its potential as signalling molecule for bacterial communication.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals or humans were used for the studies that are based on this research.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors have no conflict of interest

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