In Silico Characterization and Screening for Inhibitors of Sdia: A Protein Involved in Quorom Sensing in Escherichia Coli

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Abstract*: Bacteria use quorum sensing as a way of inter and intra- species communication. Quorum sensing was found to be important for bacteria for various processes including establishing an infection through virulence and biofilm formation. This is mediated autoinducers, which are usually produced by one group of bacteria and recognized by another group through a response regulator protein. LuxR is a response regulator protein first discovered in Vibreo fischeri and it recognizes autoinducers produced by the same species of bacteria. E. coli also has a response regulator called SdiA which is a homolog of LuxR, originally found to be involved in transcription and cell division. SdiA was later reported to regulate quorum sensing by binding to autoinducers called Acyl Homoserine Lactones (AHLs). SdiA is also reported to be involved in enhancing the multidrug resistance and virulence in pathogenic E. coli. Though many studies elaborate the functional aspects of SdiA, sequence and structural level analysis of this protein is missing in the literature. The current work aims at the in silico analysis and targeting of SdiA with structural analogs of AHLs. 7 compounds were found to be promising molecules to inhibit quorum sensing in E. coli.

***Keywords* : AHLs, SdiA, Quorum sensing.**

I. INTRODUCTION

The quorum sensing (QS) is a strategy used by many bacteria that controls gene expression in a cell density dependent manner. QS is a cell to cell communication process in which bacteria synthesize, recognize and respond to extracellular signaling molecules. QS mediates many processes in bacteria including bioluminescence, secretion of virulence factors, formation of biofilm, etc. QS mainly involves the production of chemical signaling molecules, followed by the sensing of them by the QS regulators to generate a response. The signaling molecules are referred as the autoinducers (AIs).

AIs are produced by bacteria at basal levels and their concentration increases with growth. Then these molecules diffuse through bacterial membranes and enter the cells. When reaching an optimal concentration, the autoinducer molecules interact with response regulators present inside bacterial cells and activate them, which in turn can alter gene expression. As this phenomenon happens in a cell-density-dependent manner, it is termed as quorum sensing [1-4]. QS requires AIs and response regulator proteins. AIs bind to response regulator proteins to activate it. The activated regulatory protein binds to promotor regions of genes inducing transcription of the genes [3]. Many QS systems were described based on the type of AIs and regulator proteins involved. The three main QS systems are i) LuxR/AI-1 system ii) LuxS/AI-2 system, iii) AI-3/epinephrine/norepinephrine system. *E. coli* utilizes all the three QS systems. In Lux R/AI-1 system, *E. coli* detect autoinducers from other bacteria. In LuxS/AI-2 system E. coli participate in intra and inter species signaling. During the AI-3 /epinephrine/ norepinephrine system *E. coli* recognize self produced autoinducer, or signals by other bacteria, or human stress hormones epinephrine and norepinephrine. The most intensely studied AI-1s in Gram negative bacteria are the AHLs [1]. *E. coli* can produce AI-2 molecules but not AI-1 (AHLs) [5].

Autoinducer molecules are synthesized by LuxI and its homologues by transferring a fatty acid chain from an acylated acyl carrier protein to *S*-adenosylmethionine thereby releasing the AHL and methylthioadenosine [6]. Bacteria that contain LuxI gene or its homologues can produce AHL. AHL structure contains a homoserine lactone ring linked to a fatty acid side chain. Each species of bacteria produces a distinct AHL molecule. For example, the length of acyl chain may contain four to 18 carbons, and acyl chains differ in the degree of saturation. Further, the AHL may differ at the third carbon of the acyl chain and contain a hydrogen, hyrdroxyl, carbonyl, or oxo group [4, 7, 8]. *E. coli* lacks LuxI and thus do not produce AHLs. But *E. coli* recognizes AHLs produced by other bacteria through a LuxR homolog known as SdiA [9]. SdiA detects a much broader range of AHLs than other LuxR homologs [10]. In *E. coli*, SdiA was reported to regulate biofilm formation, motility and indole production [11-13].

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SdiA mediated quorum sensing is used by *E. coli* K-12 for virulence gene expression [14] and AHL analogues are reported to inhibit the expression of virulence genes [15]. Thus it is apparent that inhibiting SdiA would be a novel way to control the virulence of *E. coli*. Interference with quorum sensing is being considered as one of the new strategies that can be regarded as an alternative to antibiotics [16]. Although SdiA has reported for its molecular mechanisms its complete structural and functional properties were unexplored. Hence, the current work aims to perform *in silico* sequence and structure analysis of SdiA and also screening for binders of SdiA that could potentially useful as regulators of quorum sensing.

II. METHODS

A. Compounds

Four AHLs which are most commonly produced in bacteria were selected from the literature (Table 4). Compounds with structural similarity with these 4 AHL molecules were retrieved using ChemMine tool [17]. 83 ligands which are similar to 4 AHL molecules were retrieved and used in this study.

B. Structure of SdiA

The crystal structure of SdiA from *E. coli* was retrieved from Protein Data Bank (PDB ID: 4Y15). It contains 246 amino acids. The structure is complexed with 3-oxo-C6-homoserine lactone.

C. Computational Details

All computations were carried out using a computer with 3GB RAM and Intel Core 2 duo E7500 processor (2.93GHz). Molecular docking studies were performed with AutoDock 4.2 running in Debian version 9. Result analysis was performed with Discovery Studio Visualizer client 4.0.

D. Sequence analysis of SdiA

Sequences of SdiA of *E. coli* K-12 and LuxR of *V. fischeri* were retrieved from Uniprot database and pair wise alignment was performed to check their identity and similarity. Also multiple sequence alignment was performed with the SdiA and LuxR family of proteins from other organisms.

E. Phylogenetic Analysis

Evolutionary history of SdiA was analyzed by constructing a Phylogenetic tree from the protein sequences of SdiA and LuxR homologs of various organisms using MEGA X [18].

F. Physicochemical Properties

Various physicochemical properties were predicted for SdiA using ExPASy-ProtParam tool [19] and subcellular localization was predicted using CELLO2GO webserver [20].

G. Structural analysis of SdiA

Structural motifs and domains present in the SdiA were analyzed using InterPro. Structure alignment of SdiA with LuxR was performed using TM-align [21] in order to understand the insights of structural similarity between these proteins.

H. Molecular Docking Studies

The protein and all the 83 ligands were prepared for docking using the graphical user interface of AutoDock tools. The preparation involved adding all hydrogen atoms to the proteins, which is a step necessary for calculation of partial atomic charges. Water molecules and heteroatoms were removed from the protein molecule [22].

By using the graphical user interface of AutoDock tools, a 3D grid box was generated to embed the protein and, Grid parameters were set. Grid maps were calculated by running AutoGrid 4. Docking parameters were set by the docking wizard of AutoDock Tools [23]. Conformation search was performed using Lamarckian Genetic Algorithm which runs for 100 cycles [24]. The binding energies for each conformation of the ligand with the proteins were determined by running AutoDock 4 [25].

Analysis of docking was performed by using the graphical user interface of AutoDock Tools and Discovery Studio Client 4.0. Binding energies of each conformation of docked compounds were noted and the best conformation was chosen based on the binding energy and number of hydrogen bonds that they form with the protein. Various types of interactions between ligand and receptor such as hydrogen bonds, hydrophobic interactions, Van der Waals and electrostatic interactions were visualized.

III. RESULTS

A. Sequence Analysis of SdiA

Sequence level identity and similarity between the SdiA of *E. coli* and LuxR of *V. Fischeri* was found to be 39% and 49% respectively. This indicates that though SdiA was reported to be a homolog of LuxR, it has a low sequence similarity with LuxR. Mulltiple sequence alignment of SdiA and LuxR of various organisms was preformed and it was found that the residues in the C- terminal regions of proteins were conserved (data not shown).

B. Phylogenetic Analysis

Phylogenetic analysis was performed with the SdiA and LuxR sequences of various organisms in order to track the evolutionary history of SdiA. (Figure 1).

C. Physicochemical Properties

The physicochemical properties such as amino acid composition, theoretical pI, extinction coefficient and instability index were obtained and listed in Table 1. The amino acid composition of this protein is shown in Table 2. The number of leucine residues in the protein is high next to alanine. Number of hydrophobic residues is high in this protein and so the aliphatic index was found to be higher.

D. Structural Analysis of SdiA

SdiA contains two domains as analyzed by InterPro, one in the N-terminal and the other in the C-terminal. The N-terminal domain is 'Winged helix-like DNA-binding domain' and the N-terminal domain is the 'Transcription factor LuxR-like, autoinducer-binding domain'. The details of these domains are presented in Table 3. Structures of LuxR and SdiA were aligned using TM-align and the

superimposed regions are shown in Figure 4.

E. Molecular Docking Studies

The ligands (n-83) were docked against the crystal structure of SdiA using AutoDock 4.2. The binding energy of each ligand and number of hydrogen bonds that they make with the protein were recorded (data not shown).

The four AHL molecules and the natural ligand that is present in the crystal structure were also docked. The binding energies of the 4 AHL molecules are presented in Table 4. The compounds showing lower binding energy than the 4 AHL molecules were considered to be top binders.

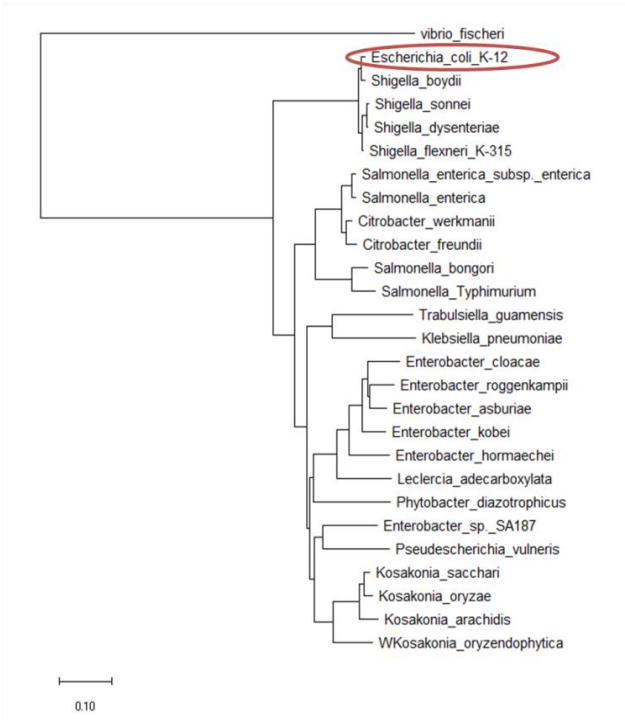


Fig. 2. Phylogenetic Tree showing the evolutionary relationship of selected SdiA and LuxR from different organisms

Table 1: Physicochemical Properties of SdiA

Molecular weight	29182.49
Theoretical pI	6.60
Total number of negatively charged residues (Asp + Glu)	29
Total number of positively charged residues (Arg + Lys)	27
Estimated half-life	30 hours (mammalian reticulocytes, in vitro) >20 hours (Yeast, in vivo) >10 hours (*Escherichia coli*, in vivo)
Instability index	46.35 This classifies the protein as unstable.
Aliphatic index	81.09
Grand average of hydropathicity (GRAVY)	-0.413

Table 2: Amino acid composition of SdiA

Amino acid	Number of residues in the protein	Percentage
Ala (A)	21	8.5%
Arg (R)	16	6.5%
Asn (N)	13	5.2%
Asp (D)	8	3.2%
Cys (C)	3	1.2%
Gln (Q)	14	5.6%
Glu (E)	21	5.5%
Gly (G)	6	2.4%
His (H)	11	4.4%
Ile (I)	11	4.4%
Leu (L)	27	10.9%
Lys (K)	11	4.4%
Met (M)	12	4.8%
Phe (F)	12	4.8%
Pro (P)	11	4.4%
Ser (S)	14	5.6%
Thr (T)	11	4.4%
Trp (W)	5	2.0%
Tyr (Y)	10	4.0%
Val (V)	11	4.4%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%

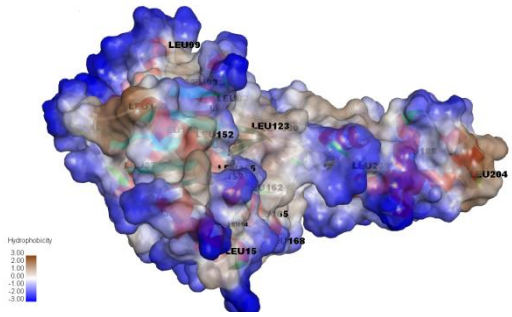


Fig. 3. Hydrophobicity map of SdiA with highlighted Leucine residues

Table 3: Domains present in SdiA

Name of the domain	Description	Amino acids
Winged helix-like DNA-binding domain superfamily	Winged helix DNA-binding proteins contain a related winged helix-turn-helix DNA-binding motif, where the "wings" are small beta-sheets. The winged helix motif consists of two wings, three alpha helices (H1, H2, H3) and three beta-sheets (S1, S2, S3) arranged in the order H1-S1-H2-H3-S2-W1-S3-W2	175 - 244

Transcription-factor LuxR-like, autoinducer-binding domain	This domain binds N-acyl homoserine lactones (AHLs). In most cases, binding of AHL by this N-terminal domain leads to unmasking of the DNA-binding domain, allowing it to bind DNA and activate transcription.	23 - 145

Based on this, the cut off value for top binders were set as -7.61 kcal/mol, which is the lowest binding energy value showed by the AHL molecule, C8. Out of the 83 compounds docked, 7 compounds showed binding energy of less than -7.61kcal/mol. (Table 5).

The compounds Beta- D- gluco- hexopyranosyluronic acid- (1->3)- 2-acetamido-2-deoxy-beta-D-gluco-hexopyranosyl- (1->4)-beta- D- gluco- hexopyranosyluronic acid-(1->3)-2-acetamido-2-deoxy- beta- D- gluco-hexopyranose (**cpd a**), Etelcalcetide, Cyclosporine, Clindamycin and Beta-D- gluco- hexopyranosyluronic acid-(1->3)-2-acetamido-2-deoxy-hexopyranosyl-(1->4)-beta-D-gluco-hexopyranosyluronic acid- (1->3)- 2- acetamido-2- deoxy- hexopyranose (**cpd b**), Clindamycin Phosphate and Glutathione were the top binders. Binding interactions of the top binder **cpd a** with SdiA is given in Figure 5. The amino acids interacting with these top 7 compounds are given in Table 5.

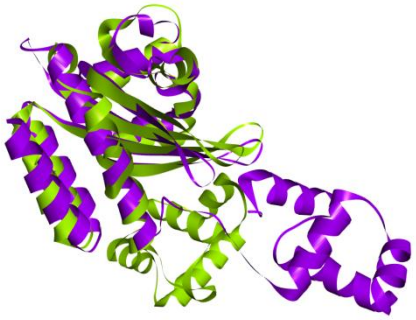


Fig. 4. Structure alignment of LuxR and SdiA proteins; Purple- SdiA; Green- LuxR

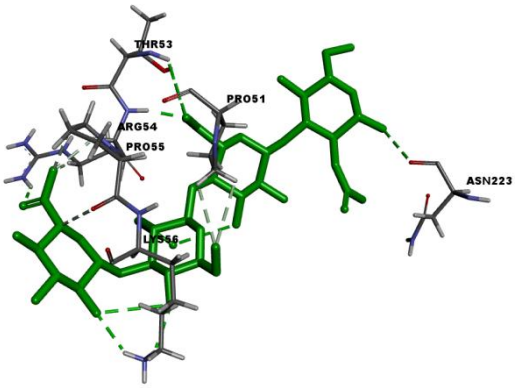


Fig. 5. Amino acids interacting with cpd a, the top compound

Table 4: List of AHL molecules selected for the study and their binding energies with SdiA

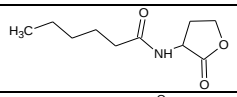
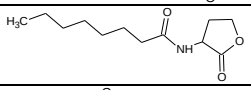
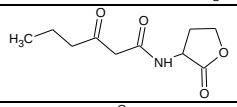
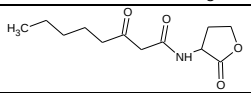
S. No	AHL	Structure	Binding Energy Kcal/mol
1.	N-hexanoyl-L-homoserine lactone (C6)		-6.81
2.	N-octanoyl-L-homoserine lactone(C8)		-7.61
3.	N-(3-oxo-hexanoyl)-L-homoserine lactone (oxo C6)		-7.08
4.	N-(3-oxo-octanoyl)-L-homoserine lactone (oxo C8)		-7.57

Table 5: Amino acids of SdiA interacting with top 7 binders

Rank	Compound	Binding Energy Kcal/mol	Interacting amino acids
1	Cpd a	-9.88	Pro51, Arg54, Pro55, Lys56, Asn223
2	Etelcalcetide	-9.56	Pro51, Arg54, Pro55, Lys56, Val57, Gln72, Pro225, Gln229
3	Cyclosporine	-9.14	Pro81, Leu83, Pro85, Glu86, Asn87, Phe88, Ser89, Leu99, Tyr122, Met124, Leu130
4	Clindamycin	-8.52	Arg54, Val57, Leu77, Ala78, Pro81, Leu83
5	Cpd b	-8.5	Asn127, Met166, Thr175, Met178, Lys182, Lys185, Arg189, Leu239, Ile240, His241, His242
6	Clindamycin Phosphate	-8.28	Met163, Met166, Lys182, Lys185,
7	Glutathione	-7.95	Lys182, Lys185, Arg189, His242

IV. DISCUSSION

Bacteria interact with their community through a group of small signaling molecules known as autoinducers. This process called as quorum sensing, involves a group of individual organisms to an environmental response. Earlier reports underscore the importance of quorum sensing in virulence and biofilm formation in bacteria [26-29].

The autoinducers are either produced by one species and recognized by the other or produced and recognized by the same species. For recognizing the autoinducers the bacteria need a response regulator protein. LuxR is such a response regulator found in many bacteria initially discovered in *V. fischeri*. *E. coli* do not produce autoinducers but it can recognize the autoinducers called AHLs through a LuxR homolog called SdiA. SdiA belongs to the LuxR family of transcriptional regulators that induce ftsQAS locus in cell division [13]. SdiA enhances multidrug resistance by stimulating efflux pumps in *E. coli* [11]. Thus SdiA could be a potential target towards pathogenic strains of *E. coli* such as uropathogenic *E. coli* and Enteropathogenic *E. coli [30]*.

Though many studies have found out the molecular mechanism of SdiA function, no reports are available on sequence and structural level comparison of LuxR with SdiA. *The current study involves in silico characterization of SdiA and screening for inhibitors using molecular docking studies. Various physicochemical parameters of SdiA were analyzed and was found that SdiA is rich in hydrophobic residues especially leucine (Figure 3). This could contribute to higher* thermal stability of the protein [31].

*The pairwise sequence alignment of SdiA and LuxR shows that they share less percentage similarity but the multiple sequence alignment of sequences of LuxR family of proteins shows that the homology between these proteins is due to the conserved residues present in the C-terminal region of the protein. This is further confirmed by the structure alignment which shows that the two domains, DNA binding domain and AHL binding domain are overlapping in Lux-R and SdiA. The w*inged helix-like DNA-binding domain consists of two wings (W1, W2), three alpha helices (H1, H2, H3) and three beta-sheets (S1, S2, S3) arranged in the order H1-S1-H2-H3-S2-W1-S3-W2. All these wings, helices and sheets are overlapping in LuxR and SdiA (Figure 4).

Molecular docking studies were performed with 83 AHL analogs as analog-based inhibition is one of the promising strategies in designing new drugs. Seven top binders were found to have lower binding energy than the AHL molecules. The amino acids Arg54, Lys182, Lys185 and His242 are found to be interacting with 3 or more top binding compounds, which suggest that these amino acids might be essential for binding. These top compounds can further be studied as inhibitors of quorum sensing using *in vitro* studies and can be developed into potential drugs

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