Molecular modeling and active site analysis of SdiA homolog, a putative quorum sensor for Salmonella typhimurium pathogenecity reveals specific binding patterns of AHL transcriptio...

ARTICLE *in* JOURNAL OF MOLECULAR MODELING · JUNE 2012

Impact Factor: 1.74 · DOI: 10.1007/s00894-012-1469-1 · Source: PubMed

3 AUTHORS:

Gnanendra Shanmugam
Mahendra Arts & Science College
9 PUBLICATIONS **7** CITATIONS
SEE PROFILE

Anusuya Shanmugam
8 PUBLICATIONS **43** CITATIONS
SEE PROFILE

Jeyakumar Natarajan
Bharathiar University
29 PUBLICATIONS **220** CITATIONS
SEE PROFILE

J Mol Model
DOI 10.1007/s00894-012-1469-1

ORIGINAL PAPER

Molecular modeling and active site analysis of SdiA homolog, a putative quorum sensor for *Salmonella typhimurium* pathogenecity reveals specific binding patterns of AHL transcriptional regulators

Shanmugam Gnanendra · Shanmugam Anusuya · Jeyakumar Natarajan

Received: 24 April 2012 / Accepted: 13 May 2012

Abstract *Salmonella typhimurium* is a Gram-negative bacterium responsible for human diseases including gastroenteritis and typhoid fever and its quorum sensing system is currently being intensively researched. Molecular modeling and binding site analysis of SdiA homolog, a putative quorum sensor of the LuxR family and responsible for *S. typhimurium* pathogenecity revealed a high structural homology of their active site with three other LuxR family proteins LasR from *Pseudomonas aeruginosa*, TraR from *Agrobacterium tumifaciens* and CviR from *Chromobacterium violaceum*. The results show that all the LuxR family proteins harbor three conserved amino acids Tryptophan (W67) and Aspartic acid (D80) for formation of hydrogen bridges and Tyrosine (Y71) for the hydrophobic interactions (corresponding to their position in *S. typhimurium* SdiA) with acyl homoserine lactones (AHL) –dependent transcriptional regulators. However, in addition to the above conserved residues, Arginine (R60) also plays an important role in *S. typhimurium* SdiA binding with its AHL auto inducers and the complex is found to be stronger because of the interactions between nitrogen atoms of Arginine with the carbonyl oxygen in the lactone ring of AHL. The specific binding patterns would be helpful in guiding both enzymatic studies as well as design of specific inhibitors to overcome *S. typhimurium* pathogenecity.

Keywords AHL auto inducers · LuxR family proteins · Quorum-sensing · *Salmonella typhimurium* · Transcriptional regulators · Co-infection

S. Gnanendra (✉)
Department of Bioinformatics, Vivekanandha College of Arts and Sciences for Women,
Tiruchengode 637 205, India
e-mail: gnani.science@gmail.com

S. Anusuya (✉)
Department of Bioinformatics, VMKV Engineering College,
Vinayaka Missions University,
Salem 636 308, India
e-mail: anushya_bioin@yahoo.co.in

S. Gnanendra · S. Anusuya · J. Natarajan (✉)
Department of Bioinformatics, Bharathiar University,
Coimbatore 641 046, India
e-mail: n.jeyakumar@yahoo.co.in

Introduction

The Gram-negative bacilli, *S. typhimurium* is the most prevalent food borne pathogen that causes enteric fever (typhoid) and gastroenteritis in human [1, 2]. The important survival strategy of *S. typhimurium* is colonization during infection. Many countries documented outbreaks associated with drug-resistant *S. typhimurium* in humans, poultry, cattle, and swine [3, 4]. Emerging antibiotic resistance of *S. typhimurium* in both humans and animals is a serious and potential public health problem [5, 6]. Many Gram-negative bacteria including *S. typhimurium* employ a cell–cell signaling system referred to as quorum sensing (QS) to control the expression of several genes in response to its population size. In *S. typhimurium* virulence gene clusters SPI1 (Salmonella pathogenicity island 1) and spv (Salmonella plasmid virulence) are under the control of population density [7]. This phenomenon is mediated by a signal molecule's N-acyl-L-homoserine lactone (AHL) often known as auto inducers which generally binds to and actives its cognate receptor (LuxR homologs) [8–10]. *S. typhimurium* encodes a LuxR homolog, SdiA (suppressor of cell division inhibition) but does not encode an AHL synthase (LuxI homolog) [11, 12]. Hence the acyl homoserine

Published online: 02 June 2012

lactone (AHL) signal molecules cannot be produced by *S. typhimurium* itself. Instead it responds to the AHL signals produced by the other bacterial species [13]. For instance, *Salmonella* SdiA is functional during co-infection with an AHL producing pathogen *Y. enterocolitica*, and the regulated genes promoted by SdiA are involved in *Salmonella*'s colonization of the intestine [14, 15].

The expression of virulence gene network by the activated SdiA is illustrated in Fig. 1. AHLs produced by other bacterial species such as *Y. enterocolitica* diffuse across the membrane and are bound by SdiA. SdiA then increases the expression of the genes found on the virulence plasmid pSLT. These genes include pefI, srgD, srgA, srgB, rck, and srgC [16, 17]. srgE (SdiA-regulated gene E) is a chromosomal gene regulated by SdiA that also appears to be found on the virulence plasmid pSLT [13]. sirA (Salmonella invasion regulator) is a response regulator and an apparent "housekeeping" also at the regulatory cascade of SdiA. The Salmonella pathogenecity island 1 (SPI1) is regulated by sirA regulating motility and invasion functions and directly regulates the activation of the hilA and hilC expression encoding a type III and type I secretion systems and csrB expression which encodes RNA-binding protein [18].

In the present study, the quorum sensing enhanced transcriptional regulator SdiA from *S. typhimurium* was chosen as a potential drug target due to its manifestation in regulating the genes involved in colonization and virulence factors and modeled using homology modeling method. In addition the molecular docking studies between AHL auto inducer and SdiA, and the other three LuxR homolog's LasR from *P. aeruginosa*, TraR from *A. tumifaciens* and CviR from *C. violaceum* were pursued to find the unique binding patterns of SdiA, which will be helpful in designing potential inhibitors for quorum sensing.

Materials and methods

Target sequence and potential template search

The quorum sensing cognate receptor of *S. typhimurium*, SdiA sequence was retrieved from the National Centre for Biotechnology Information (NCBI) [19] (NCBI protein code EFX49656). The homologous sequences were searched against PDB using NCBI-BlastP (basic local alignment search tool) [20]. The most homologous sequence obtained was considered as the potential template structure for homology modeling. The atomic co-ordinate file of the template structure was obtained from the PDB [21]. The ClustalW [22] program was used for sequence alignment and refinement of alignment errors as homology modeling relies on the sequence alignment

Homology modeling of *S. typhimurium* SdiA

The final sequence alignment file of target and the template sequence and the atomic coordinate file of the template structure was used to build the model using the automated homology modeling software Modeler9v9 [23] that generates 3D model by satisfaction of spatial restraints. A bundle of 50 models from random generation of the starting structure was calculated and among the generated models, the best model with the least RMSD value was selected by superimposing the model with its template using SUPERPOSE [24]. This model was subjected for energy minimization by applying 20 steps of each steepest descent and conjugate gradient using GROMOS [25] of SwissPDB-viewer and was used for further analysis.

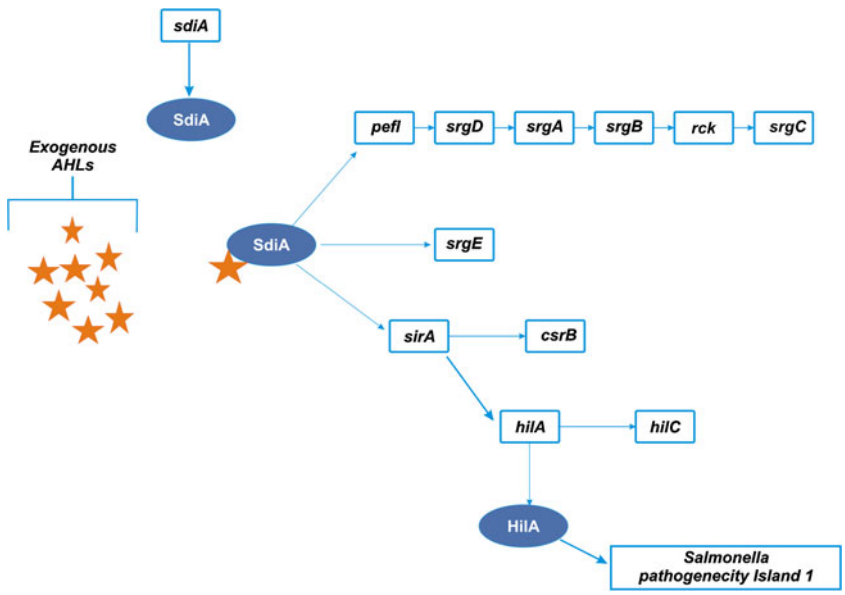

Fig. 1 The virulence gene network of *S. typhimurium* SdiA by the activated exogenous AHLs

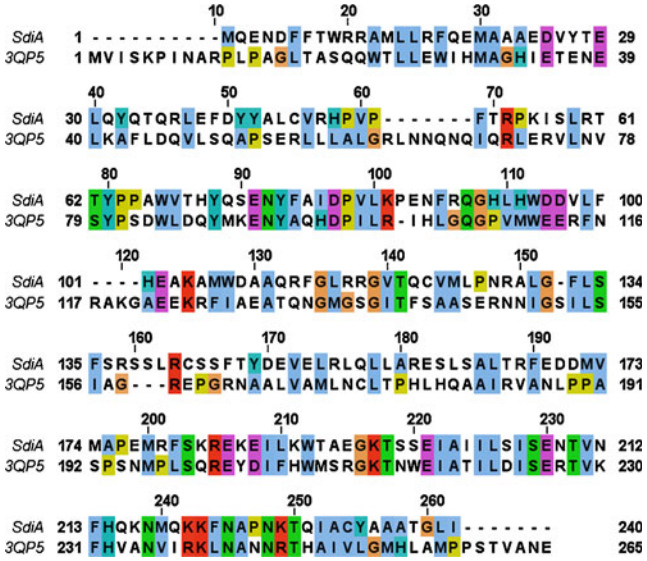

Fig. 2 Sequence alignment of modeled *S. typhimurium* SdiA and the template *C. violaceum* CviR (PDB ID : 3QP5)

Model assessment

The quality of the generated model was assessed by checking the stereo chemical parameters using PROCHECK [26], Verfiy3D [27] and ERRAT [28] at SAVES server [29]. The pdbsum [30] was used to predict the secondary structure conformations for the built model.

Prediction of conserved residues among the LuxR family proteins

The LuxR family proteins from the following four species LasR from *P. aeruginosa* (PDB ID: 2UV0), TraR from *A. tumifaciens* (PDB ID: 1L3L), CviR from *C. violaceum* (PDB ID: 3QP5) and the developed model of SdiA from *S. typhimurium* were used for the prediction of conserved residues among the LuxR proteins. Multiple sequence alignment using ClustalW [22] was carried out among these four transcriptional regulators to trace out the homology and conserved residues.

Prediction of binding site

To determine the binding affinities between *S. typhimurium* SdiA and its AHL auto inducers, the amino acids in the binding site of the developed model of SdiA was predicted through Q-site finder [31]. In addition, the binding sites of the other three LuxR family proteins LasR, TraR, and CviR from the other three species were also analyzed with their experimentally determined crystal structures with bound ligands. Finally, the binding site of SdiA was compared with binding sites of other LuxR family proteins to find the strictly conserved residues located in the active sites.

Ligand generation and flexible docking

The AHL auto inducer molecules of *Y. enterocolitica* C6-Homoserine Lactone, C8-Homoserine Lactone, 3-Oxo-C6-Homoserine Lactone and 3-oxo-C8-Homoserine Lactone were drawn in ACD-Chemsketch [32] and their SMILES notation was obtained. They were converted into SDF files using '*Online SMILES convertor and Structure file generator'* [33]. The developed SDF structures were docked with the amino acids in the binding site of *S. typhimurium* SdiA using FlexX [34] with following parameters i) default general docking informations, ii) base placement using triangle matching, iii) scoring of full score contribution and threshold of 0,30 and No score contribution and threshold of 0,70. iv) chemical parameters of clash handling values for protein ligand clashes with maximum allowed overlap volume of 2.9 A^{03} and intra-ligand clashes with clash factor of 0.6 and considering the hydrogen in internal clash tests. v) default docking details values of 200 for both the maximum number of solutions per iteration and maximum number of solutions per fragmentation.

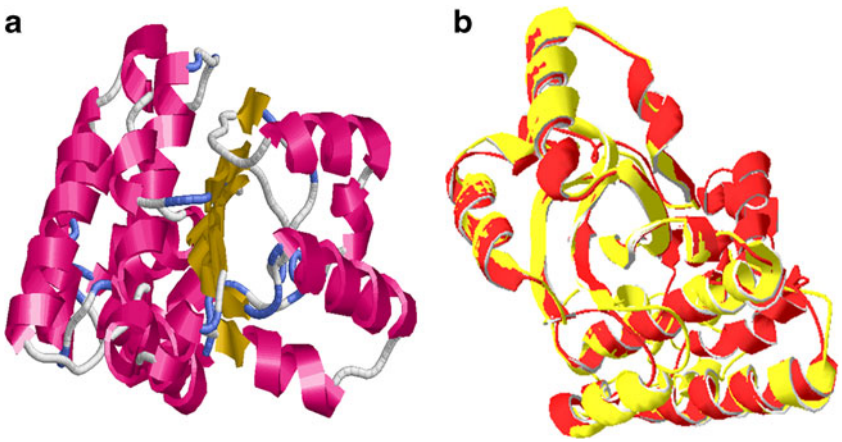

Fig. 3 **a** Ribbon diagram of the modeled *S. typhimurium* SdiA showing the α-Helices, β-Strands and loops in pink, yellow and blue respectively. **b** Superimposition of the template *C. violaceum* CviR (PDB ID : 3QP5) (red) and the generated model *S. typhimurium* SdiA (yellow)

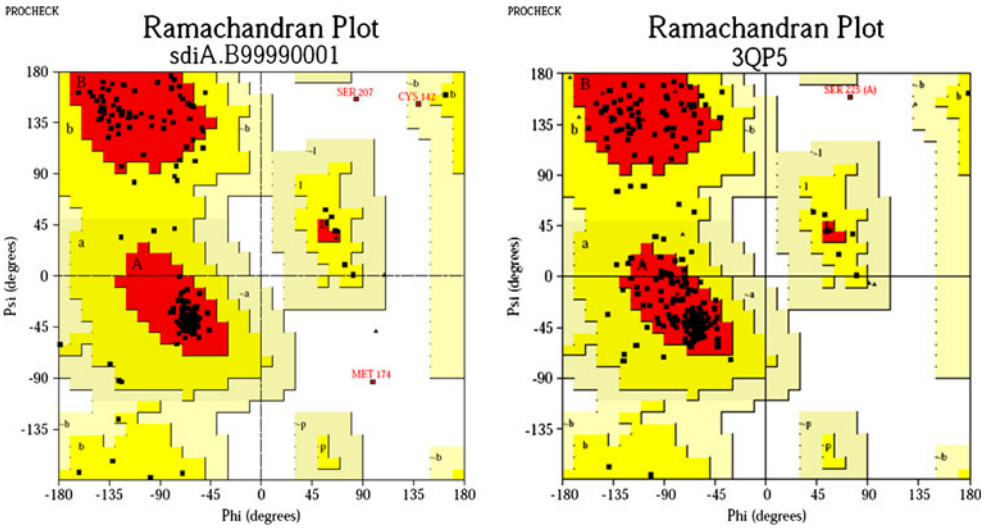

Fig. 4 Ramachandran plot for modeled *S. typhimurium* SdiA and its template *C. violaceum* CviR (PDB ID: 3QP5)

Prediction of ligand- receptor interactions

The interactions of AHL molecules with SdiA in the docked complex were analyzed by the pose-view of LeadIT [35]. The other three LuxR family proteins considered for this study, LasR, TraR, and CviR were the complexes of AHL receptors with their bound ligands and available in PDB databank [21]. LasR is a LuxR homolog bound with native ligand N-3-oxo-dodecanoyl-L-HSL. Similarly TraR is a LuxR homolog bound with its ligand 3-oxo-Octanoic acid. CviR is a LuxR homolog bound to a potential anatagonist 4-(4-chlorophenoxy)N-[(3S)-2-oxo tetrahydrofuran-3-yl butamide. The ligand receptor interactions in these LuxR homologs with the bound ligands were analyzed using the pose view of PDB Ligand Explorer [35].

Results and discussion

S. typhimurium, SdiA as drug target

S. typhimurium encode a single LuxR homolog named SdiA, but lack a corresponding LuxI homolog. The LuxI homologs in most of the Gram-negative bacteria generate the signal molecules known as AHL auto inducers. Usually these signals were detected by the LuxR homologs. However in *S. typhimurium* the LuxI homolog has not been present, indicating that it does not produce AHL of its own. Instead it responds to the signals produced by the other pathogenic bacteria such as *Y. enetrocolitica* [14, 15]. Thus *S. typhimurium* pathogenecity is most favorably expressed only during co-infection with other AHL producing pathogens. As SdiA is a transcriptional regulator of many of the virulent genes, it was considered as a potential drug target and modeled and reported in this work.

Sequence analysis and potential template for comparative modeling

The BLASTP analysis of target sequence of the transcriptional regulator SdiA from *S. typhimurium*, against PDB resulted that crystal structure of A-chain of CviR, LuxR- type transcriptional factor from *C. violaceum* (PDB ID : 3QP5) as the most homologous sequence

Table 1 Validation of the model SdiA by SAVES Server

Protein	PROCHECK					ERRAT	VERIFY-3D
	G-Factor	Ramachandran plot					
		MFR	AAR	GAR	DAR		
SdiA	- 0.15	88.7 %	9.9 %	0.5 %	0.9 %	73.216	92.62 %
CviR	- 0.12	86.5 %	13.1 %	0.0 %	0.5 %	75.309	93.25 %

MFR- Most favored region
AAR- Additionally allowed regions
GAR- Generously allowed regions
DAR- Disallowed regions

with the sequence identity of 40 % at an E-value cutoff of 4e-15. As both the sequences belong to the same LuxR family the resultant homologous sequence was selected as template structure for homology modeling. The pair wise sequence alignment of target SdiA and the template CviR was refined using ClustalW and the alignment was shown in Fig. 2.

Homology modeling of *S. typhimurium* SdiA

The sequence alignment file was used as input to build the initial model of SdiA using Modeler 9v9 by applying spatial restraints from the initial structure, a bundle of 50 models were developed using random generation and the best model was selected for further analysis based on its structural compatibility (structure with lowest DOPE score). The modeled structure was shown in Fig. 3a. The quality of the model was accessed by the results of the server SUPERPOSE. SUPERPOSE compared the predicted structure of *S. typhimurium* SdiA to crystal structure of *C. violaceum* CviR, (PDB ID : 3QP5) via superposition and was shown in Fig. 3b. The root mean square deviation (RMSD) of the modeled protein from the experimentally determined structure of *C. violaceum* CviR was found to be 0.80 A^0. As both sequences belong to LuxR family, their sequence identity (40 %) and the low RMSD values suggested that this model is valid.

The overall stereo chemical quality of the model was assessed by PROCHECK, Verfiy3D, and ERRAT of SAVES server. The Ramachandran plot of the energy minimized model of SdiA showed 88.7 % of the residues in the most favorable region, 9.9 % in the additionally allowed region, 0.5 % in the generously allowed region and 0.9 % in the disallowed region; whereas the corresponding values for the *C. violaceum* CviR, template were 86.5 %, 13.1 %, 0.0 % and 0.5 %, respectively (Fig. 4). This ensures that the generated model was good when compared with the template structure which shows only 86.5 % of residues in most favored region. The total quality G-factor was −0.15. Further the overall quality factor and compatibility of an atomic model (3D) with amino acid sequence (1D) for the modeled protein SdiA was observed as 73.216 and 92.62 % from ERRAT and Verify3D respectively. Where as in the case of template *C. violaceum* CviR the values for ERRAT and Verify3D were 75.309 and 93.25 % respectively (Table 1). The results of ERRAT and Verify-3D also confirm the model was reliable and of good quality. The secondary structure prediction for the model showed 15 Helices, 5 Strands, 10 Beta turns, 3 Gamma turns and 3 Beta hairpins (Fig. 5) indicating that the protein belongs to class alpha + beta.

Conservation amino acid residues in active site region of SdiA and other LuxR family proteins

The validated and the final model of *S. typhimurium* SdiA was then used for finding conserved and active site residues in SdiA and similar LuxR homolog proteins from other species to evaluate its binding patterns with AHL auto inducer molecules. The sequences of three LuxR homologs, LasR from *P. aeruginosa* (PDB ID: 2UV0), TraR from *A. tumifaciens* (PDB ID: 1L3L), CviR from *C. violaceum*

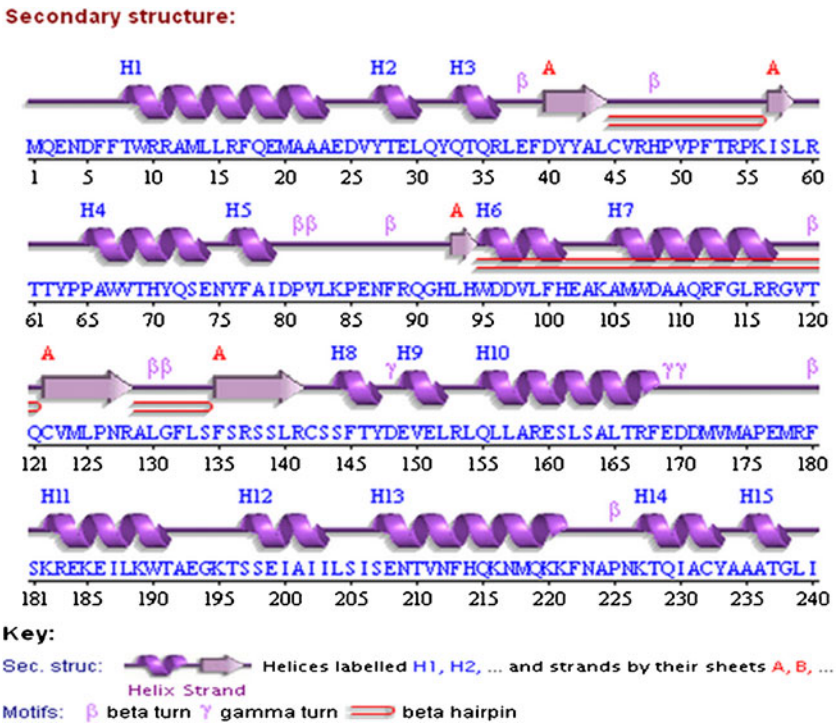

Fig. 5 Predicted secondary structure for the *S. typhimurium* SdiA. The secondary structural elements α-helices, β-strands and loops are shown above the alignment

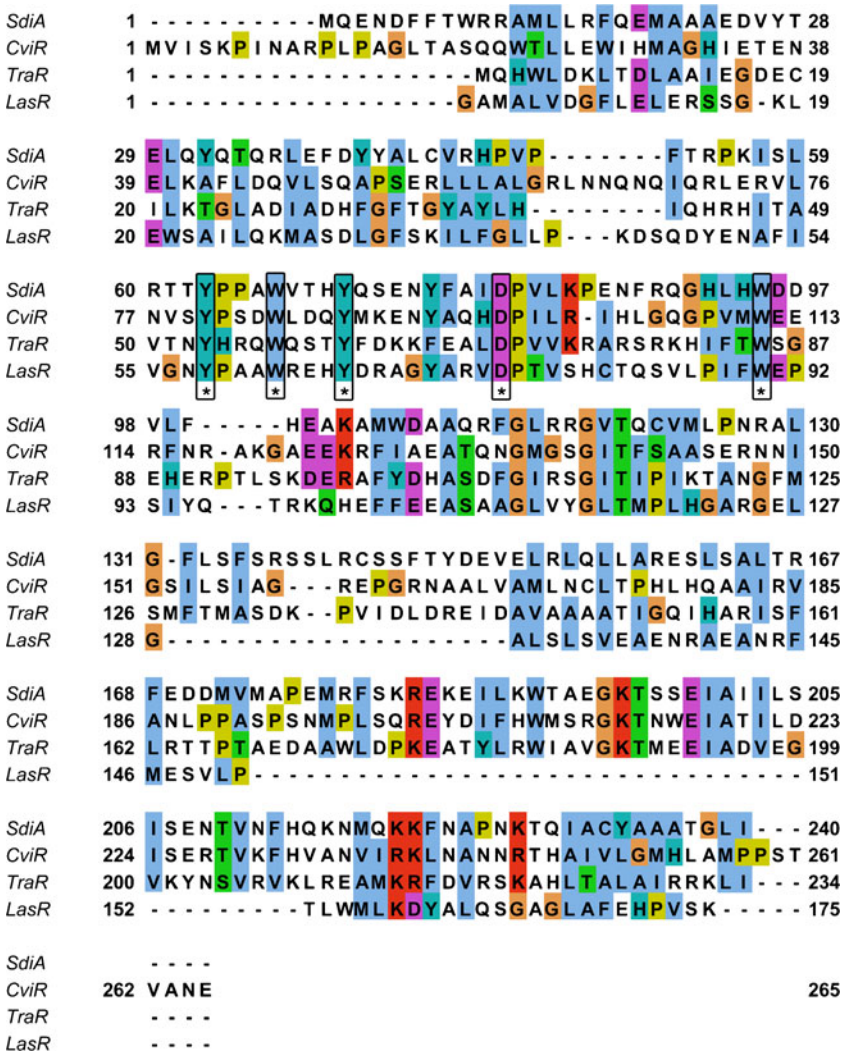

Fig. 6 Multiple sequence alignment of LuxR type family proteins *P. aeruginosa* LasR, *A.tumifaciens* TraR, *C. violaceum* CviR and *S. typhimurium* SdiA. The conserved amino acids found in the active site are indicated in boxes

(PDB ID: 3QP5) were aligned with the SdiA of *S. typhimurium* using the ClustalW program and the final alignment was optimized manually. The residues invariant across LuxR homologs were illustrated in Fig. 6.

Prediction of the binding site of *S. typhimurium* SdiA was carried-out using Q-site finder, which yields putative amino acid residues in the binding pocket. Similarly, the binding pocket for the other three LuxR proteins, LasR, TraR and CviR, whose crystal structures were already available in PDB database were obtained using ligand explorer [35]. The conserved amino acids observed all four LuxR proteins with their binding site residues and are shown in Table 2.

Table 2 The conserved amino acids of the LuxR family proteins LasR, TraR, CviR and SdiA

LasR *(P. aeruginosa)*	TraR *(A. tumifaciens)*	CviR *(C. violaceum)*	SdiA *(S. typhimurium)*
Tyr 56[a]	Tyr 53[a]	Tyr 80	Tyr 63
Trp 60[a]	Trp 57[a]	Trp 84[a]	Trp 67[a]
Tyr 66[a]	Tyr 61[a]	Tyr 88[a]	Tyr 71[a]
Asp 73[a]	Asp 70[a]	Asp 97[a]	Asp 80[a]
Pro 76	Pro 71	Pro 98	Pro 81
Trp 90	Trp 85	Trp 111	Trp 95
Ala 107	Ala 105	Ala 130	Ala 110
Gly 111	Gly 109	Gly 134	Gly 114
Gly 115	Gly 113	Gly 138	Gly 118
Thr 117	Thr 115	Thr 140	Thr 120

[a]Residues bind with ligands

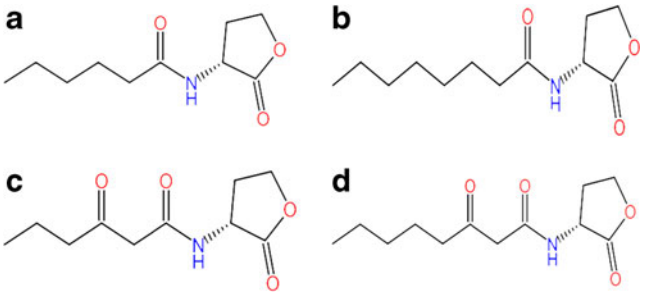

Fig. 7 Structures of AHLs produced by *Y. enterocolitica* **a** C6-homoserine lactone **b** C8-homoserine lactone **c** 3-oxo-C6-homoserine lactone **d** 3-oxo-C8-homoserine lactone

Docking studies with AHL auto inducers

Dyszel et al. [14] reported that the *Salmonella* SdiA is functional only during the co-infection of AHL producing pathogens. The studies of Throup et al. [36] reported that SdiA is consistent with specific preference to AHLs *N*-hexanoyl homoserine lactone (C6-HSL), *N*-(3-oxohexanoyl) homoserine lactone (3-oxo-C6-HSL), *N*-octanoyl homoserine lactone (C8-HSL) and *N*-(3-oxo-octanoyl) homoserine lactone (3-oxo-C8-HSL) produced by *Y. enetrocolitica*. These four AHL autoinducers, C6-HSL, 3-oxo-C6-HSL, C8-HSL and 3-oxo-C8-HSL (Fig. 7) from *Y. enterocolitica* were docked with modeled SdiA and also with its template CviR from *C.violaceum* using the docking program FlexX and the ligand-receptor interactions were analyzed using LeadIT. The other three LuxR homologous proteins, LasR from *P. aeruginosa*, TraR from *A. tumifaciens* and *CviR* from *C. violaceum* were also docked with their native ligands N-3-oxo-dodecanoyl-L-HSL, 3-oxo-Octanoic acid and 4-(4-chlorophenoxy)N-[(3S)-2-oxo tetrahydrofuran-3-yl butamide respectively and the binding interactions were studied.

The docking interactions of the exogenous AHL molecules with modeled SdiA and its template CviR(3QP5) implies that the AHL molecule 3-oxo-C6-HSL has highest binding energy of -12.991 kJ mol^{-1} and -14.548 kJ mol^{-1} respectively and C8-HSL showed lowest binding energy of -9.615 kJ mol^{-1} and -11.066 kJ mol^{-1} respectively. This observation suggests that the folding of SdiA would be the same as its template protein CviR and Table 3 summarizes these results.

Figures 8 and 9 show the docking results of SdiA and the other three LuxR homologous proteins and Table 4 shows

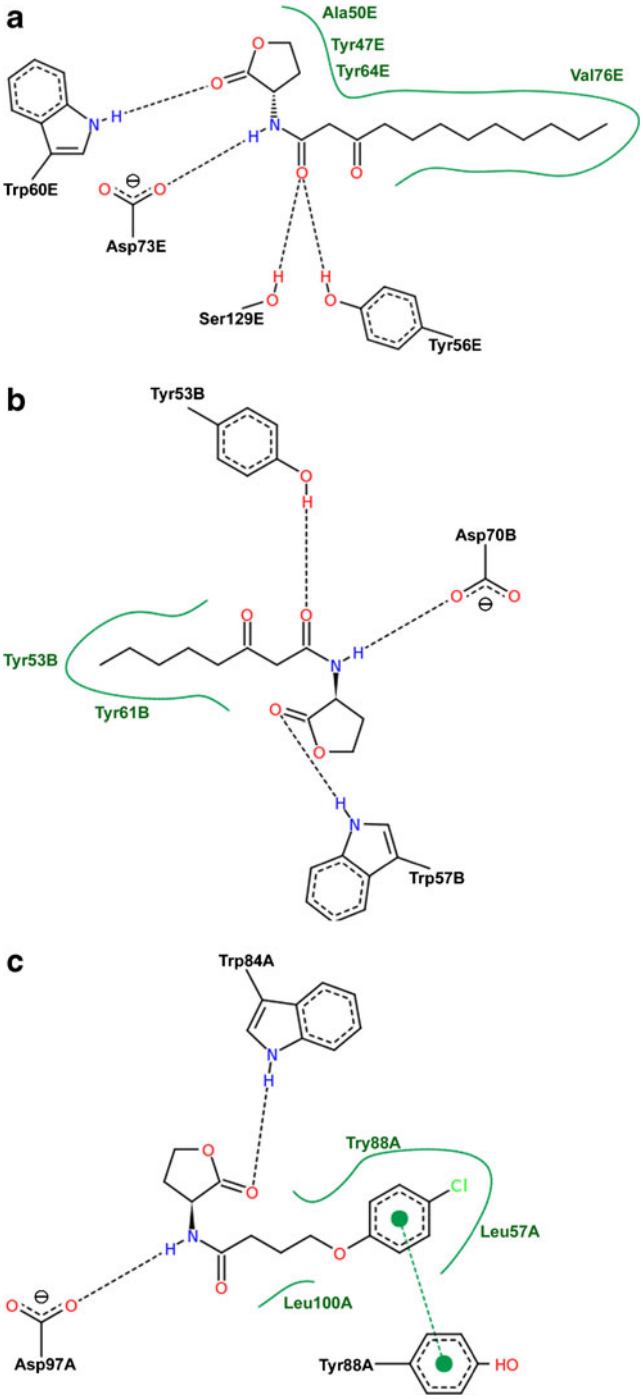

Fig. 8 Amino acid involved in binding of the native ligands to their respective cognate receptors **a** LasR bound with N-3-oxo-dodecanoyl-L-HSL **b** TraR bound with 3-oxo-Octanoic acid (**c**) CviR bound with 4-(4-chlorophenoxy)N-[(3S)-2-oxo tetrahydrofuran-3-yl butamide

Table 3 Binding energies of docked ligands (produced by *Yersinia enterocolitica*) between modeled SdiA and the template CviR (3QP5)

Receptors	Ligands with binding energies (kJ/mol)			
	C6-HSL	C8-HSL	3-oxo-C6-HSL	3-oxo-C8-HSL
SdiA(predicted model) *S. typhimurium*	-12.490	-9.615	-12.991	-11.426
CviR (3QP5-template) *C.violaceum*	-13.924	-11.066	-14.548	-11.596

the corresponding amino acids with their specific binding energies favoring the interactions. The binding energy scores of the three LuxR homologous were distributed closely from -19.33 kJ mol^{-1} to -20.76 kJ mol^{-1} and in the case of SdiA from -9.61 kJ mol^{-1} to -12.99 kJ mol^{-1}. The binding energy scores of AHL auto inducers with SdiA showed greater variability when compare to the other three LuxR homologous.

The overall docking results of AHL molecules with LuxR family proteins discloses the importance of lactone ring and the alkyl chains in AHL molecules in forming most of the interactions with the residues in the active site of LuxR family protein. The amino acids that interacted with AHL molecules were found to be Y56, W60, D73, S129 in LasR, Y53, W57, D70 in TraR and W84, Y88, D97 in CviR. These findings suggested that the conserved Tryptophan (W), Aspartic acid (D) and Tyrosine(Y) in the active site of LuxR family proteins are crucial for AHL binding.

Similar binding interactions were also observed in the docking studies of *S. typhimurium* SdiA with AHL molecules. The conserved amino acids Tryptophan (W67), Aspartic acid (D80), Tyrosine (Y71) and Arginine (R60) were involved in the AHL interaction (Fig. 9a–d). This result indicates that in *S. typhimurium* SdiA, in addition to the involvement of essential residues Tryptophan, Aspartic acid and Tyrosine in binding with AHL molecules, Arginine (R60) is also found to be necessary in favoring the interactions.

The docking studies of *S. typhimurium* SdiA and other LuxR family proteins with their respective AHL molecules implies that the electro negative element present in the lactone ring of AHL binds with Aspartic acid in the active site of LuxR family proteins. Whereas the carbonyl oxygen present in the lactone ring of AHL prefers to bind with Tyrosine residues in the active site. In SdiA, in addition to these interactions, the complex is found to be stronger because of the electrostatic and hydrogen bonding interactions between two nitrogen atoms of Arginine with the carbonyl oxygen in the lactone ring of AHL.

Conclusions

The *S. typhimurium* transcriptional regulator SdiA, involves in the cross-chemical communication with different pathogens which leads to co-infection. The AHLs produced by

Table 4 Binding residues of LuxR family proteins LasR, TraR, CviR and SdiA with their corresponding ligands

Protein	LasR *(P. aeruginosa)*	TraR *(A. tumifaciens)*	CviR *(C. violaceum)*	SdiA *(S. typhimurium)*			
Bound ligand	Lig[a]	Lig[b]	Lig[c]	Lig[d]	Lig[e]	Lig[f]	Lig[g]
	-	-	-	-	-	Tyr 41	Tyr 41
	Tyr 47	-	-	-	-	-	-
	Ala 50	-	-	-	-	-	-
	Tyr 56	Tyr 53	-	-	-	-	-
	-	-	-	Arg 60	Arg 60	Arg 60	Arg 60
	-	-	Leu 57	-	-	-	-
	Trp 60	Trp 57	Trp 84	Trp 67	Trp 67	Trp 67	Trp 67
	Tyr 64	Tyr 61	Tyr 88	Tyr 71	Tyr 71	Tyr 71	Tyr 71
	Asp 73	Asp 70	Asp 97	Asp 80	Asp 80	Asp 80	Asp 80
	Val 76	-	-	-	Val 82	-	-
	-	-	-	-	Leu 83	-	-
	-	-	-	Trp 95	Trp 95	Trp 95	Trp 95
	-	-	Leu 100	-	-	-	-
	-	-	-	-	Val 119	-	-
	Ser 129	-	-	-	-	-	-
Binding energy (kJ/mol)	-19.3133	-19.5402	-20.7605	-12.490	-9.615	-12.991	-11.426

Lig[a] - N-3-oxo-dodecanoyl-L-HSL

Lig[b] - 3-oxo-octanoic acid

Lig[c] - 4-(4-chlorophenoxy)N-[(3S)-2-oxo tetrahydrofuran-3-yl butamide

Lig[d] - C6-HSL

Lig[e] - C8-HSL

Lig[f] - 3-oxo-C6-HSL

Lig[g] - 3-oxo-C8-HSL

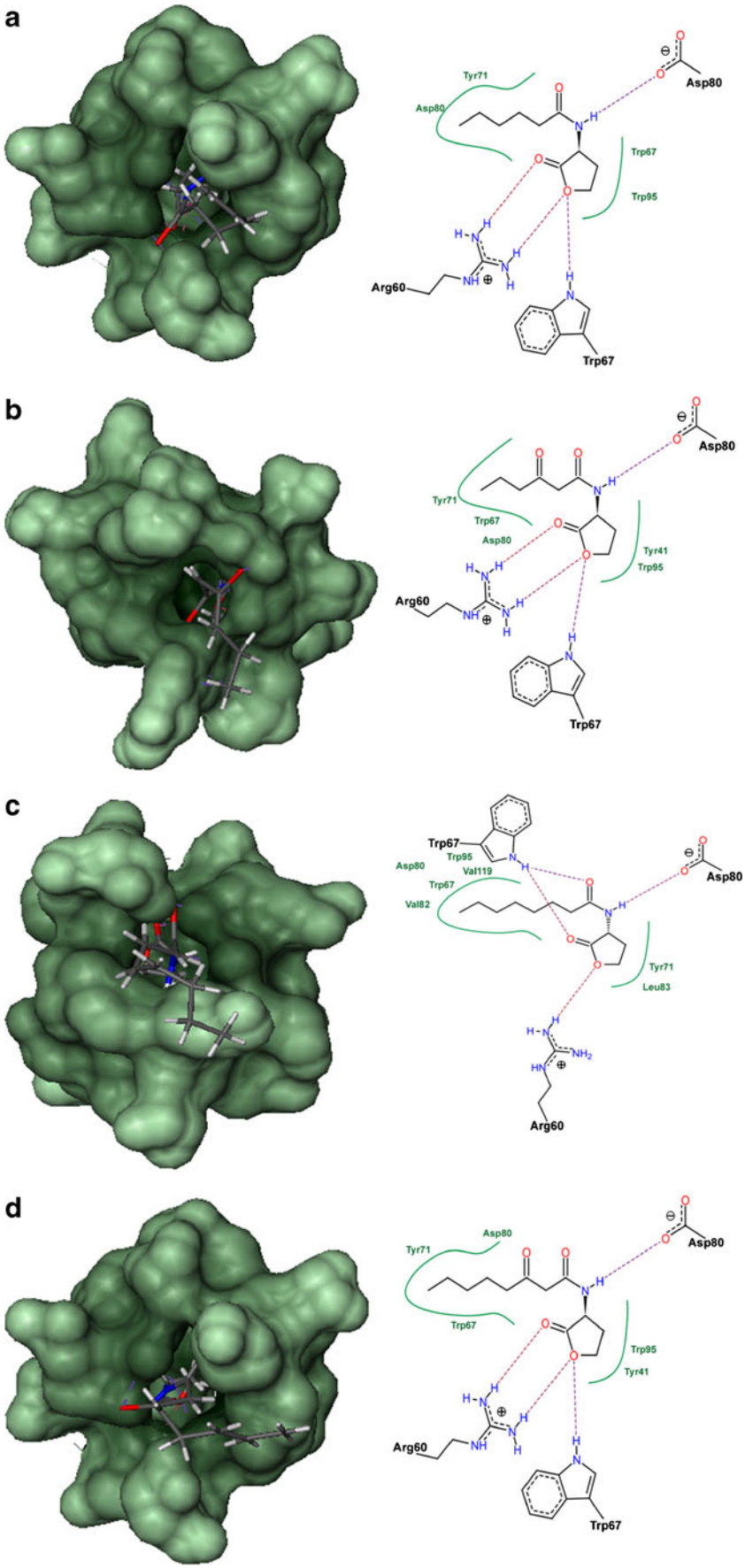

Fig. 9 Docking of *Y. enterocolitica* AHL molecules with *S. typhimurium* SdiA and the amino acid binding patterns **a** C6-HSL docked with SdiA **b** 3-oxo- C6-HSL docked with SdiA **c** C8-HSL docked with SdiA **d** 3-oxo-C8-HSL docked with SdiA

the *Y. enterocolitica* (C6-HSL, C8-HSL, oxo-C6-HSL and oxo-C8-HSL) binds to *Salmonella* SdiA to regulate the genes involved in pathogenicity, which are downstream to SdiA. Hence, SdiA is considered as a valid drug target. The binding mode of the AHL with SdiA might explain the potentiality of AHL analogous as quorum sensing inhibitors. Based on this, a model of SdiA was generated by comparative modeling and was validated. The binding mode of the AHLs was evaluated by docking studies. This work imparted that the conserved amino acids in the active site of LuxR family proteins Aspartic acid (D), Tryptophan (W) and Tyrosine (Y) were involved in the binding of AHL. The interaction is favored by the bond formation with lactone ring and hydrophobic interaction with the alkyl chain of AHL. However, in SdiA, in addition to these interactions Arginine (R60) is also found to be crucial in favoring the interactions and the complex is found to be stronger because of the electrostatic and hydrogen bonding interactions between two nitrogen atoms of Arginine with the carbonyl oxygen in the lactone ring of AHL. The present model and the binding features form the basis for further molecular studies on *S. typhimurium* SdiA quorum sensing inhibitor design which can serve as an alternate strategy for the treatment of diseases caused by *S. typhimurium*.

References

1. Scherer CA, Miller SI (2001) Molecular pathogenesis of *salmonellae*. In: Groisman EA (ed) Principles of bacterial pathogenesis. Academic, New York, pp 266–333
2. Grein T, O'Flanagan D, McCarthy T, Bauer D (1999) An outbreak of multidrug-resistant *Salmonella typhimurium* food poisoning at a wedding reception. Ir Med J 92:238–241
3. Davies A, O'Neill P, Towers L, Cooke M (1996) An outbreak of *Salmonella typhimurium* DT104 food poisoning associated with eating beef. Commun Dis Rep CDR Rev 6(11):R159–R162
4. Molbak K, Baggesen DL, Aarestrup FM, Ebbesen JM, Engberg J, Frydendahl K, Gerner-Smidt P, Petersen AM, Wegener HC (1999) An outbreak of multidrug-resistant, quinolone-resistant *Salmonella enterica serotype typhimurium* DT104. N Engl J Med 341 (19):1420–1425
5. Cloeckaert A, Chaslus-Dancla E (2001) Mechanisms of quinolone resistance in *Salmonella*. Vet Res 32:291–300
6. Piddock LJ (2002) Fluoroquinolone resistance in *Salmonella serovars* isolated from humans and food animals. FEMS Microbiol Rev 26:3–16
7. Coombes BK, Coburn BA, Potter AA, Gomis S, Mirakhur K, Li Y, Finlay BB (2005) Analysis of the contribution of *Salmonella* pathogenicity islands 1 and 2 to enteric disease progression using a novel bovine ileal loop model and a murine model of infectious enterocolitis. Infect Immun 73:7161–7169
8. Marketon MM, Gronquist MR, Eberhard A, Gonzales JE (2002) Characterization of the *Sinorhizobium meliloti* sinR/sinI locus and the production of novel N-acyl homoserine lactone. J Bacteriol 184:5686–5695
9. Parsek MR, Greenberg EP (2000) Acyl-homoserine lactone quorum sensing in Gram-negative bacteria: a signaling mechanism involved in associations with higher organisms. Proc Natl Acad Sci USA 97:8789–8793
10. Steindler L, Venturi V (2007) Detection of quorum-sensing N-acyl homoserine lactone signal molecules by bacterial biosensors. FEMS Microbiol Lett 266:1–9
11. Ahmer BMM, Jv R, Timmers CD, Valentine PJ, Heffron F (1998) *Salmonella typhimurium* encodes an SdiA homolog, a putative quorum sensor of the LuxR family, that regulates genes on the virulence plasmid. J Bacteriol 180:1185–1193
12. Janssens JC, Metzger K, Daniels R, Ptacek D, Verhoeven T (2007) Synthesis of N-acyl homoserine lactone analogues reveals strong activators of SdiA, the *Salmonella enteric serovar Typhimurium* LuxR homologue. Appl Environ Microbiol 73:535–544
13. Smith JN, Ahmer BM (2003) Detection of other microbial species by *Salmonella*: expression of the SdiA regulon. J Bacteriol 185:1357–1366
14. Dyszel JL, Smith JN, Lucas DE, Soares JA, Swearingen MC, Vross MA, Young GM, Ahmer BM (2010) *Salmonella enteric serovar Typhimurium* can detect acyl homoserine lactone production by *Yersinia enterocolitica* in mice. J Bacteriol 192:29–37
15. Smith JN, Dyszel JL, Soares JA, Ellermeier CD, Altier C, Lawhon SD, Adams LG, Konjufca V, Curtiss R III, Slauch JM, Ahmer BM (2008) SdiA, an N-acylhomoserine lactone receptor, becomes active during the transit of *Salmonella enteric* through the gastrointestinal tract of turtles. PloS ONE 3:e2826
16. Nicholson B, Low D (2000) DNA methylation-dependent regulation of pef expression in *Salmonella typhimurium*. Mol Microbiol 35:728–742
17. Michael B, Smith JN, Swift S, Heffron F, Ahmer BM (2001) SdiA of *Salmonella enteric* is a LuxR homolog that detects mixed microbial communities. J Bacteriol 183:5733–5742
18. Jiménez-Gómez P, Pozuelo de Felipe MJ, Llinares Pinell F, García de los Ríos JE (2007) Quorum-sensing in *Pseudomonas aeruginosa* and *Salmonella*: active natural compounds as antagonists. Commun Curr Res Edu Topics Trends App Microbiol 1:41–51
19. NCBI. http://www.ncbi.nlm.nih.gov/
20. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410
21. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE (2000) The protein data bank. Nucl Acids Res 28:235–242
22. Thompson JD, Higgins DG, Gibson TJ (1994) Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting position- specific gap penalties and weight matrix choice. Nucl Acids Res 22:4673–4680
23. Sali A, Blundell TL (1993) Comparative protein modelling by satisfaction of spatial restraints. J Mol Biol 234:779–815
24. Maiti R, Van Domselaar GH, Zhang H, Wishart DS (2004) SuperPose: a simple server for sophisticated structural superposition. Nucleic Acids Res 32(1):W590–W594
25. Walter RP, Scott PH, Hunenberger IG, Tironi AE, Mark SR, Billeter JF, Torda AE, Huber T, Kruger P, van Gunsteren WF (1999) The GROMOS biomolecular simulation program package. J Phys Chem A 103(19):3596–3607
26. Laskowski RA, MacArthur MW, Moss DS, Thornton JM (1993) PROCHECK a program to check the stereo chemical quality of protein structure. J Appl Cryst 26:283–291
27. Luthy R, Bowie JU, Eisenberg D (1992) Assessment of protein models with three-dimensional profiles. Nature 356:83–85
28. Colovos C, Yeates TO (1993) Verification of protein structures: patterns of nonbonded atomic interactions. Protein Sci 2:1511–1519
29. SAVES (2011) http://nihserver.mbi.ucla.edu/SAVES

30. Laskowski RA, Chistyakov VV, Thornton JM (2005) PDBsum more: new summaries and analyses of the known 3D structures of proteins and nucleic acids. Nucleic Acids Res 33:D266–D268
31. Laurie AT, Jackson RM (2005) Q-SiteFinder: an energy-based method for the prediction of protein-ligand binding sites. Bioinformatics 21:1908–1916
32. ACD/ChemSketch Freeware, version 11 (2006) Advanced Chemistry Development, Inc. Toronto, ON, Canada, www.acdlabs.com
33. Weininger D (1988) SMILES, a chemical language and information system. Introduction to methodology and encoding rules. J Chem Inf Comput Sci 28:31–36
34. Rarey M, Kramer B, Lengauer T, Klebe G (1996) A fast flexible docking method using an incremental construction algorithm. J Mol Biol 261:470–89
35. Stierand K, Maab P, Rarey M (2006) Molecular complexes at a glance: automated generation of two-dimensional complex diagrams. Bioinformatics 22:1710–1716
36. Throup JP, Camara M, Briggs GS, Winson MK, Chhabra SR, Bycroft BW, Williams P, Stewart GS (1995) Characterisation of the yenI/yenR locus from *Yersinia enterocolitica* mediating the synthesis of two nacylhomoserine lactone signal molecules. Mol Microbiol 17:345–356