www.gbtrp.com
International Journal of Biological Technology (2011) 2(3):33-37
ISSN: 0976 – 4313

Original Article

In silico Analysis of Transcriptional Regulators of *Pseudomonas aeruginosa* and *Burkholderia cepacia* responsible for Quorum Sensing

S.Jayanthi[1], B.Sharmila[2] and *Palvannan Thayumanavan[3]

[1]Department of Biotechnology, AVS College of Arts and Science, Salem - Tamil Nadu, India.
[2]Department of Microbiology, K.R. College of Arts and Science, Kovilpatti, Tamilnadu, India.
[3]Department of Biochemistry, Periyar University, Salem, Tamil Nadu, India.
*Corresponding Author: Email: pal2912@gmail.com; Mobile No.: +91-9894280618

Received: 11 .10.2011; Revised: 30.11.2011; Accepted: 01.12.2011; Published: 15.12.2011.

Abstract

Quorum sensing is the process where bacteria respond to high intracellular concentrations of Autoinducers, which bind to their cognate receptors to regulate the transcription of many virulence genes in many gram negative bacteria including the opportunistic pathogens *Pseudomonas aeruginosa* and *Burkholderia cepacia*. These were an important pathogen in patients with cystic fibrosis. Twenty percent of cystic fibrosis patients infected with *B. cepacia* suffer from cepacia syndrome, a necrotizing pneumonia with fever and occasionally bacteremia. Hence in the present study the 3D structures of the quorum sensing enabled transcriptional activators, LasR from *Pseudomonas aeruginosa* and CepR from *Burkholderia cepacia* was generated and the molecular insights was anlaysed by comparative modeling and the their biding sites were analysed. Thus the molecular insight of these modeled structures might serve as a novel point of intervention for de novo design on therapeutic antibiotics.

Key words: Quorum sensing, Comparative Modeling, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, Trancriptional Regulators, Autoinducers

Introduction

The regulatory mechanism that controls the cell density-dependent expression of many bacterial phenotypes is known as a phenomenon named Quorum sensing (Swift *et al.*,1996). Quorum sensing is the process where bacteria respond to high intracellular concentrations of Autoinducers, which bind to their cognate receptors to regulate the transcription of selected genes. This process was first reported in *Vibrio fischeri* that controls the bioluminescence (*lux*) phenotype in the marine organism (Engebrecht *et al.,* 1984). In *V. fischeri*, the two components necessary for the expression of cell density-dependent *lux* was LuxR and LuxI proteins (Eberhard *et al.,* 1986). The LuxI protein synthesizes the autoinducer *N*-(3-oxohexanoyl)-L-HSL (Fuqua *et al.,* 1996). When these freely diffusible signaling molecule are sufficient in amounts, they binds to LuxR, which activates the *lux* genes. The threshold concentration of autoinducer necessary for the induction of bioluminescence is attained when cultures achieve a sufficiently high cell density.

This Quorum sensing phenomenon has been shown to regulate the production of virulence factors in several gram-negative species, including the opportunistic pathogens *Pseudomonas aeruginosa* and *Burkholderia cepacia* (Govan *et al.,* 1996). Quorum sensing in *P. aeruginosa* involves two unique systems, *lasRI* and *rhlRI* (Brint and Ohman, 1995). The *las* system is composed of the transcriptional activator LasR and the autoinducer *N*-(3-oxododecanoyl)-L-HSL (Pearson *et al.,* 1995). LasR activates the expression of elastase (*lasB*), alkaline protease (*aprA*), LasA protease (*lasA*), exotoxin A (*toxA*), the type II secretion apparatus (*xcpP* through *xcpZ*) and the autoinducer synthase *lasI* (Passador *et al.,* 1993 and Pearson *et al.,* 1997). The *rhl* system is composed of the RhlR transcriptional activator and the autoinducer *N*-butyryl- L-HSL (Pesci *et al.,* 1997). RhlR activates the expression of rhamnolipids (*rhlAB*), elastase (*lasB*), lipase (*lipA*), the stationary phase sigma factor gene *rpoS*, and other genes (Ochsner and Reiser, 1995). These two systems form a hierarchical quorum sensing cascade in which LasR regulates the expression of *rhlR*.

Burkholderia cepacia (previously *Pseudomonas cepacia*) is an important pathogen

www.gbtrp.com
International Journal of Biological Technology (2011) 2(3):33-37
ISSN: 0976 – 4313

in patients with cystic fibrosis (Lonon *et al.,* 1988). Twenty percent of cystic fibrosis patients infected with *B. cepacia* suffer from cepacia syndrome, a necrotizing pneumonia with fever and occasionally bacteremia (McKevitt *et al.,* 1989). This condition leads to a rapid and fatal pulmonary decline and is a unique clinical outcome in comparison to respiratory infections with other pathogens. Most cystic fibrosis patients infected with *B. cepacia* are coinfected with *P. aeruginosa*. Due to the genetic conservation of quorum-sensing regulatory elements and similarities in the structure of *N*-acyl-HSLs, the potential for cell-to-cell communication between different species exists. *B. cepacia* produces several extracellular virulence factors, including protease, lipase, and four types of siderophores(Sokol *et al.,* 1992): salicylic acid, ornibactin, pyochelin(Sokol *et al.,* 1986), and cepabactin (Meyer *et al.,* 1989). *B. cepacia* often infects the patients those are already colonized with *P. aeruginosa*. The exoproducts of *P. aeruginosa* modifie the epithelial cell surface of the lung to facilitate the attachment of *B. cepacia.* Hence in the present study, we report the modeled structure of LuxR homologs, CepR and *N*-octanoyl- HSL autoinducers of *B. cepacia* and the structure of LasR bound to its autoinducer from *P.aeruginosa*.

Materials and Methods

The quorum sensing cognate receptor CepR from *B. cepacia* and LasR from Pseudomonas aeruginosa was retrieved from the National Centre for Biotechnology Information (NCBI http://www.ncbi.nlm.nih.gov/). The homologous entries were predicted by running NCBI-Blastp (Basic Local Alignment Search Tool) (Altschul *et al.,* 1990) against protein data bank and obtained the suitable templates for comparative modeling. The proteins models of CepR and LasR were generated by using SWISS-MODEL (Guex and Peitsch 1997), an automated homology modeling server, used to model the protein (Arnold *et al.,* 2003). The Quality of the model was assessed by checking the stereochemical parameters (Ramachandran plot) using Procheck (Laskowski *et al.,*1993). The conserved regions among the amino acid Sequences of transcriptional regulators of LasR and CepR from *P. aeruginosa and B. cepacia* respectively and the used template TraR were traced out by a multiple sequence alignments, using clustalW (Thompson *et al*.,1994) at the EBI server. To determine the interactions between AHL-LasR and AHL-CepR from *P.aeruginosa and B.cepacia*, the binding pocket for the model was predicted through the server WHATIF (Vriend,1990). The multiple sequence alignment was used to trace out the conserved residues in the binding pockets of the transcriptional regulators of the developed models CepR and LasR from *B. cepacia and P. aeruginosa* respectively.

Result and Discussion

The quorum sensing receptors CepR (AAD12726) and LasR (BAA06489) from *B.cepacia* and *P. aeruginosa* was retrieved from the National Centre for Biotechnology Information (NCBI) respectively. The homologous entries obtained as templates for CepR and LasR were 3SZT and 1L3L respectively. The molecular insights of quorum sensing dependent transcriptional regulators have been well studied in many Gram negative bacteria (Persson *et al.,* 2005). These studies revealed that LuxR family proteins lead to dimerisation and DNA complex formation to regulate the transcription of many virulent genes by interacting with LuxI family proteins derived auto-inducers. The structure of this complex has been crystallised and resolved in the Agrobacterium sps, TraR (1L3L) protein bounded with the auto-inducer 3-oxo-octanoyl-L-homoserine lactone (AI-1) (Zhang *et al.,* 2002).

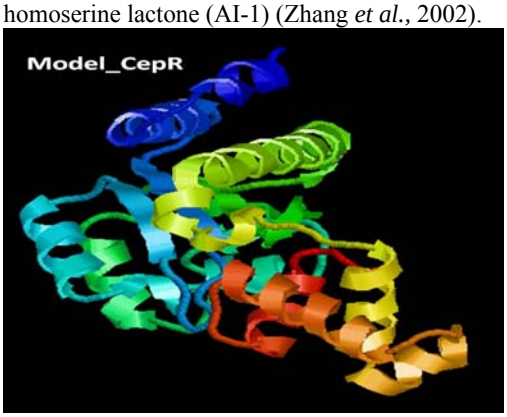

Figure.1. Modeled structure of CepR B.cepacia

The structure analysis of this protein shows that the active site is located between four a-helix and five b-sheets, constituents of the protein receiver domain. Analysis of the multiple sequence alignment shows that residues located in the active site and interacting with the ligand are strictly conserved in 1l3L, CepR and LasR. This observation ensures that the folding of CepR

www.gbtrp.com
International Journal of Biological Technology (2011) 2(3):33-37
ISSN: 0976 – 4313

and LasR should be the same as the proteins TraR, to preserve similar interactions with the ligand. Based on the protein sequence alignment of the three proteins with clustal W, the models of the protein CepR (Figure.1) and LasR (Figure.3) were generated from the structure of 3SZT and 1L3L respectively. The ramachandran plot of the energy minimized model of CepR exhibited 87.9% of the residues in the core regions (Figure.2).

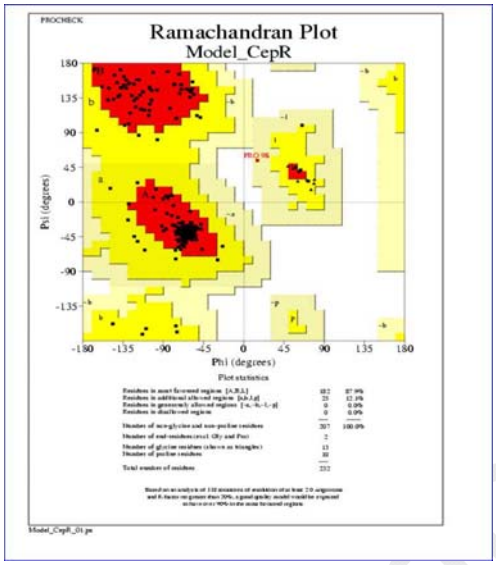

Figure. 2: Stereochemical properties of Modeled Protein CepR from *B.cepacia*

The total quality G-factor was -0.03, which indicates a good quality model (acceptable values of the G-factor in procheck are between 0 and −0.5, with the best models displaying values close to zero). The sterochemical properties of LasR model exhibited 91.3% of the residues in the core regions (Figure.4) with G-factor value of -0.04. The results of stereo chemical properties of Models and templates were compared and reported in Table1.

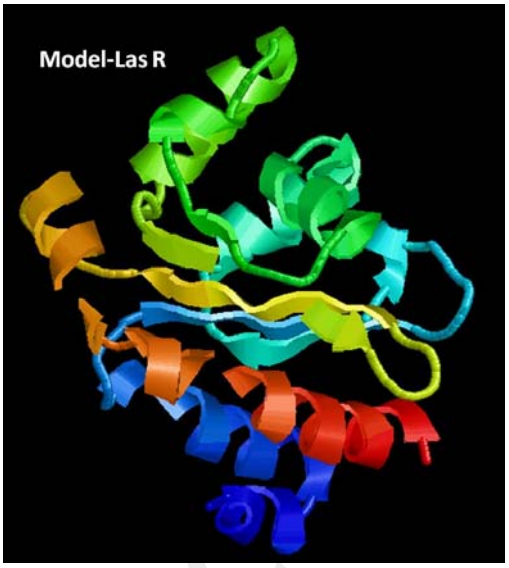

Figure.3. Modeled structure of LasR from *P. aeruginosa*

The structural similarity between the modeled LasR structure of *P. aeruginosa* and the template structure 1L3L was observed, as the RMS backbone value of 0.8A^0 and RMS value between CepR model and template 3SZT was observed as 0.74 A^0. The high structural conservation is reflected by the low RMS value of backbone superimposition. (for best models RMS value are between 0 to 1A^0). The homology between the proteins belonging to the family LuxR type (quorum sensing enhanced transcriptional regulators) used in this study were analysed by multiple sequence alignment (Fig.5) and observed that the amino acid favouring ligand interactions in 1L3L protein is conserved in the both proteins CepR and LasR of *B. cepacia* and *P. aeruginosa* (Table-2) respectively. The binding pocket for the energy minimized model of was predicted using docking options from whatif server and obtained the amino acids favoured in the binding pockets of CepR (Fig. 6) and LasR (Fig.7).

Table -1: Stereo chemical quality check by Ramachandran plot

Regions of Amino acid Residues in plot	CepR	3SZT	LasR	1L3L
Most favored regions	87.9%	87.5%	91.3%	91.2%
Additional allowed regions	12.1%	12.3%	8.7%	8.5%
Generously allowed regions	0.0%	0.0%	0.0%	0.4%
Disallowed regions	0.0%	0.0%	0.0%	0.0%
Total number of residues	239	456	239	234

www.gbtrp.com
International Journal of Biological Technology (2011) 2(3):33-37
ISSN: 0976 – 4313

Table -2: Conserved amino acids observed in multiple sequence alignment might favour ligand interactions (with reference to 1L3L crystal structure)

LuxR homologs	Conserved Amino acids
CepR	Y58, W62, Y66, D75, W90
LasR	Y56, W60, Y64, D73, W88
1L3L	Y53, W57, Y61, D70, W85

Table -3: Amino acids observed in predicted Binding Site

LuxR homologs	Predicted Binding site Amino acids
CepR	Cys38, Tyr39, Gly40, Ile41, Arg42, Val52, Ile54, Tyr66, Ile72, Asp75, Thr77, Val78, Arg79, Gly81, Ala82, Ser117, Ser118, Phe125, Gly126, Leu127, Leu128, Ser129.
LasR	Leu36, Phe37, Gly38, Leu39, Leu40, Tyr47, Glu48, Asn49, Ala50, Phe51, Ile52, Tyr56, Trp60, Arg61, Tyr64, Asp65, Ala67, Gly68, Trp69, Ala70, Arg71, Asp73, Pro74, Thr75, Val76, Cys79, Thr80, Trp88, Ile92, Tyr93, Phe101, Phe102, Ala105, Leu110, Val111, Leu114, Thr115, Leu125, Gly126, Ala127, Ser129

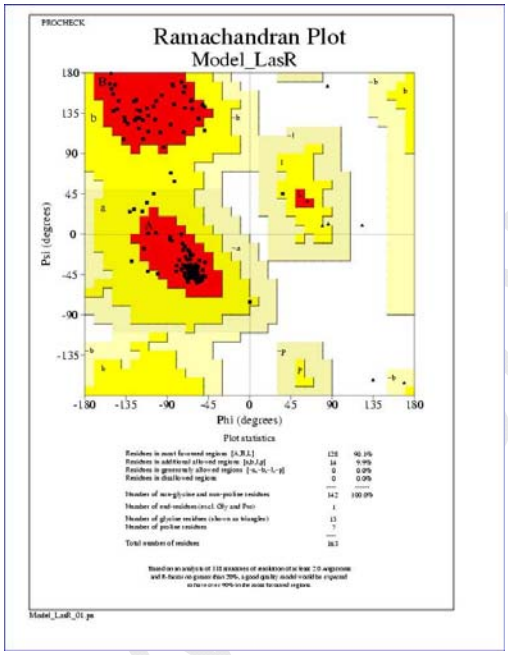

Figure. 4: Stereochemical properties of Modeled Protein LasR from *P. aeruginosa*

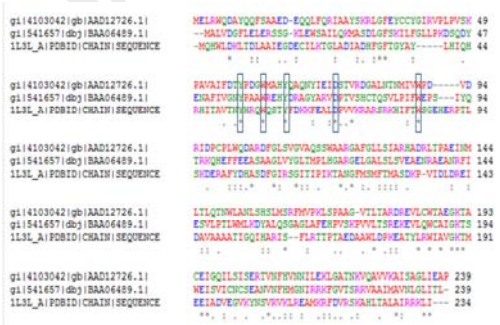

Figure 5. Multiple Sequence alignment

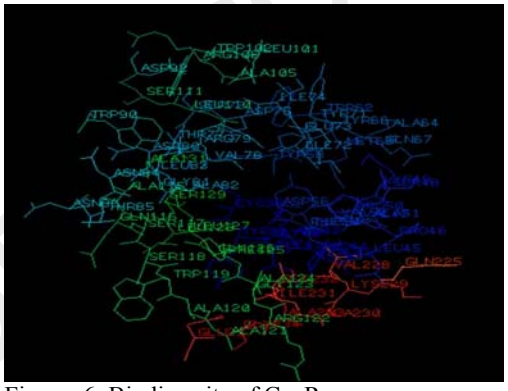

Figure. 6: Binding site of CepR

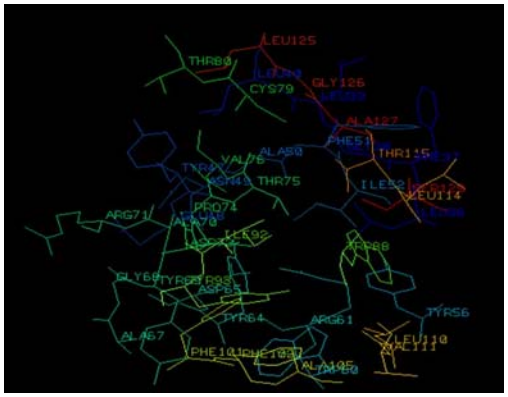

Figure. 7: Binding site of LasR

The binding pockets of all the predicted amino acids that may favour in the ligand interactions were reported in Table- 3. Thus the modeled structures of transcriptional activators LasR from *P. aeruginosa* and CepR from *B. cepacia* that controls the Quorum-sensing enabled virulence gene expression represent a

novel point of intervention for the development of new generation antibiotics.

References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D.J. 1990. Basic local alignment search tool. *J. Mol. Biol*., 215:403–410.

Arnold, K., Bordoli,L., Kopp. and Schwede, T. 2006.The SWISS-MODEL work space- A web based environment for protein structure homology modeling.*Bioinformatics*.,22:195-201.

Brint, J. M., and Ohman, D. E. 1995. Synthesis of multiple exoproducts in *Pseudomonas aeruginosa* is under the control of RhlR-RhlI, another set of regulators in strain PAO1 with homology to the autoinducer-responsive LuxR-LuxI family. *J. Bacteriol*., 177:7155–7163.

Eberhard, A., Widrig, C. A., McBath, P. and Schineller, J.B. 1986. Analogs of the autoinducer of bioluminescence in *Vibrio fischeri*. *Arch. Microbiol*.,146:35–40.

Engebrecht, J., and Silverman, M. 1984. Identification of genes and gene products necessary for bacterial bioluminescence. *Proc. Natl. Acad. Sci*. USA, 81:4154–4158.

Fuqua, C., Winans, S. C., and Greenberg, E. P. 1996. Census and consensus in bacterial ecosystems: the LuxR-LuxI family of quorum-sensing transcriptional regulators. *Annu. Rev. Microbiol*., 50:727–751.

Govan, J.R. W., Hughes, J.E. and Vandamme, P. 1996. *Burkholderia cepacia*: medical, taxonomic and ecological issues. *J. Med. Microbiol*.,45:395–407.

Guex N and Peitsch MC. 1997. SWISS MODEL and the Swiss pdb viewer: an environment for comparative protein modeling. *Electrophoresis*, 18:2714–2723.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. 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.

Laskowski, R.A., MacArthur, M.W., Moss, D.S. and Thornton, J.M. 1993. PROCHECK a program to check the stereo chemical quality of protein structure. *J. Appl. Cryst*., 26:283–291.

Lonon, M. K., Woods, D. E. and Straus, D. C. 1988. Production of lipase by clinical isolates of *Pseudomonas cepacia*. *J. Clin. Microbiol*., 26:979–984.

McKevitt, A. I., Bajaksouzian, S., Klinger, J. D., and Woods, D.E. 1989. Purification and characterization of an extracellular protease from *Pseudomonas cepacia*. *Infect. Immun*., 57:771–778.

Meyer, J., Hohnadel, M.D. and Halle, F. 1989. Cepabactin from *Pseudomonas cepacia*, a new type of siderophore. *J.Gen. Microbiol*., 135:1479–1487.

Ochsner, U. A., and Reiser, J. 1995. Autoinducer-mediated regulation of rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci*. USA, 92:6424–6428.

Passador, L., Cook, J.M., Gambello, M.J., Rust, L. and Iglewski, B. H.1993. Expression of *Pseudomonas aeruginosa* virulence genes requires cell-to-cell communication. *Science*, 60:1127–1130.

Pearson, J. P., Pesci, E.C. and Iglewski, B.H. 1997. Roles of *Pseudomonas aeruginosa las* and *rhl* quorum-sensing systems in control of elastase and rhamnolipid biosynthesis. *J.Bacteriol*., 179:5756–5767.

Pearson, J. P., Passador, L., Iglewski, B.H. and Greenberg, E.P. 1995. A second *N*-acylhomoserine lactone signal produced by *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci*. USA, 92:1490–1494.

Pesci, E. C., Pearson, J. P. Seed, P.C. and Iglewski, B.H. 1997. Regulation of *las* and *rhl* quorum sensing in *Pseudomonas aeruginosa*. *J. Bacteriol.*, 179: 3127–3132.

Zhang,R.G., Pappas, T., Brace, J.L., Miller, P.C., Oulmassov, T., Molyneaux, J.M., Anderson, J.C., Bashkin, J.K., Winans. S.C. and Joachimiak, A. 2002. Structure of a bacterial quorum-sensing transcription factor complexed with pheromone and DNA, *Nature*, 417 : 971-974.

Sokol, P. A. 1986. Production and utilization of pyochelin by clinical isolates of *Pseudomonas cepacia*. *J. Clin. Microbiol*., 23:560–562.

Sokol, P. A., Lewis, C. J. and Dennis, J. J. 1992. Isolation of a novel siderophore from *Pseudomonas cepacia*. *J. Med. Microbiol*., 36:184–189.

Swift, S., Throup, J.P., Williams, P., Salmond, G. P.C. and Stewart, G.S. 1996. Quorum sensing a population-density component in the determination of bacterial phenotype. *Trends Biochem. Sci*., 21:214–219.

Persson,T., Givskov, M. and Nielsen, J. 2005. Quorum sensing inhibition: targeting chemical communication in gram-negative bacteria. *Curr. Med. Chem.*, 12: 3103–3115.

Vriend G .1990. WHATIF: a molecular modeling and drug design program. *J. Mol. Graph*., 8:52-56.