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Identification of the Role of LuxS in the Regulation of Motility & the Expression of the Flagellar Structural & Functional Regulators in *Vibrio Cholerae*

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Keywords : *Vibrio cholerae O139 Bengal strain MO10 lac-,* Δ *luxS,* Δ *flaAluxS, motility,* Δ *-galactosidase assay.*

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Identification of the Role of LuxS in the Regulation of Motility & the Expression of the Flagellar Structural & Functional Regulators in *Vibrio Cholerae*

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Abstract - In *Vibrio cholerae* motility has an important role in virulence. Beside this *flrA*, *flrB* and *flrC* regulate the expression of both flagellar structure and function in *Vibrio cholerae*. Quorum sensing autoinducer molecules regulate the expression of EPS in *Vibrio cholerae* O139 Bengal strain MO10. The present study was conducted to investigate whether *luxS* gene (responsible for the synthesis of quorum sensing autoinducer molecule 2 i.e, AI-2) regulates motility as well as expression of both structural and functional regulatory genes *flrA, flrB* and *flrC*. In the peresent study we found that mutation in *luxS* gene caused reduction in motility and expression of flra was significantly decreased in the *luxS* mutant strain of the *Vibrio cholerae* O139 Bengal strain MO10 lac $^{-}$. However expression of *flrB* and *flrC* remained unaltered by luxs gene. So *luxS* gene may regulate motility through the upregulation of *flrA* in *Vibrio cholerae* O139 Bengal strain MO10.

Keywords : Vibrio cholerae O139 Bengal strain MO10 lac-, Δ luxS, Δ flaAluxS, motility, Δ -galactosidase assay.

I. Introduction

Vibrio cholerae is a natural inhabitant of the aquatic environment and this organism is introduced into human populations through the ingestion of contaminated food or water. In human after colonization to the small intestine through the action of a type IV pilus (TCP) it expresses cholera toxin (CT), which causes the electrolyte imbalance and profuse watery diarrhea. In natural aquatic environment Vibrio cholerae forms biofilm for its long survival in the environment and motility plays an important role in biofilm formation by inducing initial attachment to abiotic surface (Mois et al., 2009). in the planctonic state, flagella and fimbriae are crucial for motility and initial adherence to a solid surface (Watnic and Kolter 1999).

Vibrio cholerae is a highly motile organism due to presence of a polar flagellum. Motility and virulence have been inferred to be inversely related to each other (Lospalluto and Finkelstein, 1972; Mekalanos et al., 1983; Taylor et al., 1987; Richardson, 1991). But the exact role of motility in pathogenesis is still remained unclear (Prouty et al., 2001).

The flagellum is a complex structure comprising of multiple structural subunits and expression of flagellar genes is also well regulated involving a four-tiered flagellar transcription hierarchy. Three regulatory genes, *flrABC* (express flagellar regulatory proteins ABC) are additionally required for the flagellar synthesis. FlrA encoded by class I gene is the master regulator of the flagellar transcriptional hierarchy and σ^{54}-dependent transcriptional activator of class II genes, which encode mainly the MS ring and export apparatus components and regulatory factors, including FlrC (Millikan et al., 2003). FlrC is aσ^{54}-dependent transcriptional activator that is phosphorylated by FlrB (sensor kinase) component and activates class III flagellar genes which encode the basal body and hook, as well as some of the switch and export apparatus components and the FlaA flagellin. Suggestion is there that the *Vibrio cholerae* polar flagellar filament is composed of five flagellins, but only one of these, FlaA is essential for motility (Klose and Mekalanos, 1998a).

Repression of FlrA and FlrC increase expression of EPS in response to some environmental signals (Watnic et al., 2001). On the other hand in *Vibrio fishery* FlrA / FlrC are also found to be involved in the motility (Millikan et al., 2003). In the epidemic subset of *Vibrio cholerae* including some O1 El Tor and O139 strain absence of flagellar structure triggers the expression of exopolysaccharie which is required for the development of the mature biofilm (Yildiz et al., 1999; Watnic et al., 2001). Beside this *flaA* (responsible for the synthesis of flagellar structural core protein) mutant strain is nonmotile as well as defective in intestinal colonization (Watnick et al., 2001).

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It was previously proposed that cross-species quorum-sensing signaling molecule AI-2 (*luxS* gene product) stimulates biofilm formation and alters its architecture by stimulating flagellar motion and motility in *Escherichia coli* (González-Barrios et al., 2006). Moreover *luxS* gene was also reported to be involved in controlling chemotaxis, flagellar synthesis and motility in *E. coli* and *Helicobactor pylori* (Ren et al., 2004; Sperandio et al., 2001; Osaki et al., 2006).

It is also observed that *luxS* gene (responsible for the synthesis of quorum sensing cell signaling molecule autoinducer 2 i.e AI-2) regulates EPS expression and biofilm formation in *Vibrio cholerae* O139 Bengal strain MO10 lac$^-$ through the Quorum sensing autoinducers and flagellum-dependent parallel but converging EPS signaling circuits independent of input of LuxO-HapR (Biswas et al., 2012, unpublished). However expression of *flaA* gene (responsible for the synthesis of flagellar structural core protein) was not under the control of *luxS* gene (Biswas et al., 2012, unpublished). So, on the basis of these reports the present study was carried out to investigate whether *luxS* gene (responsible for the synthesis of quorum sensing autoinducer molecule 2 i.e, AI-2) regulates motility as well as expressions of both structural and functional regulatory genes *flrA, flrB* and *flrC*.

II. Methods and Materials

a) Bacterial growth

MO10 *luxS*, *flaAluxS* mutant strain as well as *Vibrio cholerae* O139 Bengal strain MO10 lac$^-$, a clinical isolate and a strain with high epidemiological importance were used for the present investigation. *Vibrio cholerae* O139 Bengal strain MO10 lac$^-$, *luxS*, *flaAluxS* were grown in Luria-Bertani (LB) broth, supplemented with streptomycin (100 μg of per ml). All the strains used for this study were displayed in the Table 2.

b) Detection of Motility

Motility of the *Vibrio cholerae* strains were tested by the method mentioned by Rasid et al. (2003) using swarm plate containing 0.3 % LB agar.

c) Plasmid construction

The promoter-*lacZ* fusion containing transcriptional reporter plasmid of *flrA, flrB* & *flrC* were prepared by PCR amplification of the respective promoter using primer pairs Promoter A and B for corresponding gene (Table 1). PCR generated fragment was digested with EcoRI and BamHI and ligated into the corresponding sites of plasmid vector pRS551 (Simons et al., 1987). Then finally electroporated into *Vibrio cholerae* MO10 lac$^-$ wild type, ***ΔluxS*** and ***ΔflaAluxS*** strains. All plasmid constructs and strains used in this study were displayed in Table 2. All PCRs were performed using MO10 chromosomal DNA as template and specific primer pairs which were designed depending on the specific gene sequence from complete *Vibrio cholerae* genome sequence (Kwok et al., 1990; Heidelberg et al., 2000). Chromosomal DNA (template DNA) was isolated by the method of Sambrook et al., 2001.

d) β -Galactosidase assays

Vibrio cholerae MO10 lac$^-$ wild type, ***ΔluxS*** and ***ΔflaAluxS*** strains were transformed with plasmids pSH130, pSH131 and pSH132 respectively, the transcriptional reporter constructs (Table 2). Bacterial cells grown in LB broth were harvested at OD_{600} of ~ 0.2 to 0.4, permeabilized with chloroform and sodium dodecyl sulfate and assayed for β-galactosidase activity following the method mentioned by Miller et al., 1992.

Table 1: Oligonucleotide primer sequence

Target gene or encoding region	Primer sequence (5'-3')
FlrA Promoter-A	GC GAATTCGCTCTAGATAGTTTCGCTAA
FlrA Promoter-B	GCGGATCCGCTCTAGACAAGCGAACCAT
FlrB Promoter-A	GCGAATTCGCAGATCTTGGGTTGGCTTC
FlrB Promoter-B	GCGGATCCGCAGATCTCAGCCAGAGCCT
FlrC Promoter-A	GC GAATTC GCCACTCATAACCCGCTAAA
FlrC Promoter-B	GCGGATCC CCAGAGTCGTTGCAGCACAA

Table 2 : Plasmid and strains used in this study

Strains	Description	Source
Vibrio cholerae strains		
MO10 lac$^-$	WildType,O139 Bengal strain	Laboratory collection
CG104	MO10 lac$^-$ Δ*luxS*	This study
CG110	MO10 lac$^-$ *ΔflaAluxS*	This study
Plasmids		
pRS551	Transcriptional *lacZ* fusion vector; Amp^r	Laboratory collection
pSH130	*flrA-lacZ* in pRS551	This study
pSH131	*flrB-lacZ* in pRS551	This study
pSH132	*flrC-lacZ* in pRS551	This study

III. Result and Discussion

In *Vibrio cholerae* flagellar synthesis and motility are thought to be important for cholera pathogenesis, but the exact role they play in virulence is still not completely understood. (Lospalluto and Finkelstein, 1972; Mekalanos et al., 1983; Taylor et al., 1987; Richardson et al., 1991; Prouty et al., 2001).

In many bacterial species, flagellar gene transcription occurs in a regulatory hierarchy in which the expression of late genes (i.e., class III or IV) is dependent on the expression of early ones (i.e., class I or II). In *Vibrio cholerae*, the σ^{54}-dependent transcriptional activator FlrA is the sole class I gene which regulates the expression of FlrABC regulon (Prouty et al., 2001; Josenhans et al., 2002; Stewart et al., 1996). FlrA, FlrB and FlrC are reported to be additionally required for the expression of both flagellar structure and function in *Vibrio cholerae*. The flagellar motor regulates the exopolysaccharide expression. EPS expression was reported to be involved in biofilm formation and this in turn also affects the virulence of the organism because motility was required for its behavior of colonization (Klose & Mekalanos, 1998a). Report shows that repression of either FlrA- or FlrC-dependent transcription ceases flagellar synthesis and produces rugose colony in wild type *Vibrio cholerae* O139 (Watnic et al., 2001). Mutations in flagellar genes at any level results in the absence of a complete flagellar filament and also produce rugose colony morphology in the *Vibrio cholerae* O139 strain MO10 (Watnic et al., 2001).

Quorum sensing cell signaling molecule autoinducer 2 (*luxS* gene product) is also previously reported to control EPS expression, biofilm formation as well as motility in *Helicobactor pylori* as well as *E. Coli* (Ren et al., 2004; Osaki et al., 2006; Sperandio et al., 2001,

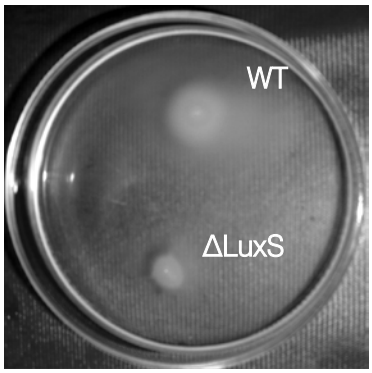

Fig.1 : Comparision of motility between Wild type & Δ*luxS* strain

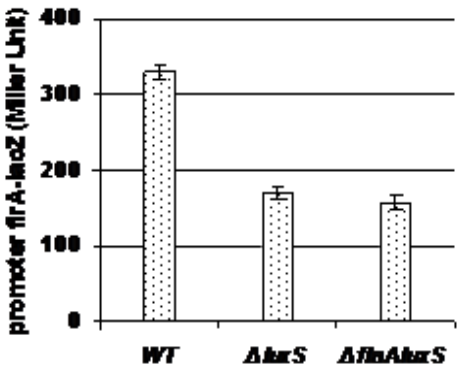

Fig. 2 : Comparison of *flrA* expression among *luxS* mutant strain of *Vibrio cholerae* MO10 lac$^-$ & wild type Transcription level of *flrA* was observed by Δ -galactosidase assay using *flrA promoter-lacZ* fusion transcriptional reporter construct

Additionally it was also proposed that *luxS* gene (responsible for the synthesis of quorum-sensing signal AI-2) is involved in the regulation of EPS expression as well as biofilm formation in *Vibrio cholerae* O139 Bengal strain MO10 lac $^-$ (Biswas et al., 2012 unpublished). So, on the basis of this background we were interested to investigate the role of *luxS* (responsible for the synthesis of quorum sensing autoinducer molecule 2 i.e, AI-2) in motility as well as in the expression of both flagellar structural and functional regulatory genes i.e *flrA, flrB* and *flrC*. In our study we found that motility was reduced in the *luxS* mutant strain of *Vibrio cholerae* O139 Bengal strain MO10 lac$^-$ than that of the wild type (fig-1). Beside this we also found that expression of *flrA* is significantly decreased after deletion in *luxS* gene in *Vibrio cholerae* MO10 lac $^-$ O139 Bengal strain (Fig 2). But expression of

flrB, flrC were not significantly altered in *luxS* mutant strains (Fig 3 &Fig 4). So, these observations suggested that *luxS* gene (responsible for the synthesis of quorum sensing autoinducer molecule 2 i.e, AI-2) upregulated the trancription of *flrA*, but transcription level of *flrB* and *flrC* were not influenced by *luxS* gene. Finally it can also be proposed that *luxS* gene may regulate the motility through the upregulation of expression of *flrA* in this epidemic subset of *Vibrio cholerae*.

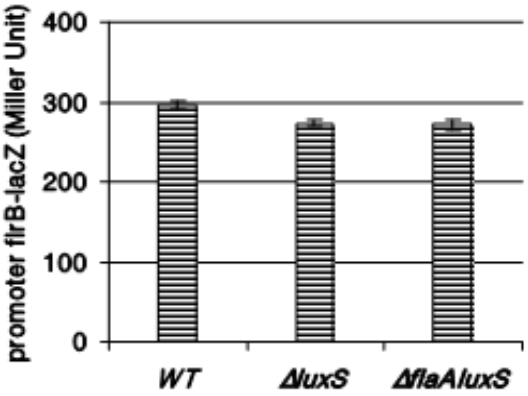

Fig.3 : Comparison of *flrB* expression among *luxS* mutant strain of *Vibrio cholerae* MO10 lac⁻ & wild type Transcription level of *flrB* was examined by β-galactosidase assay using *flrB promoter-lacZ* fusion transcriptional reporter construct

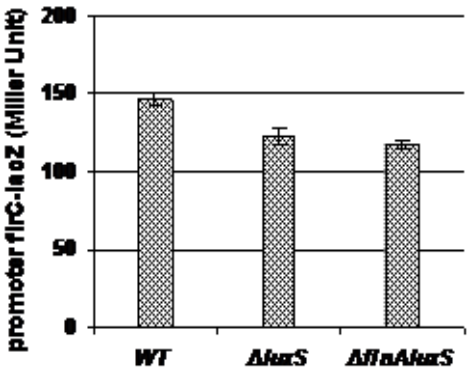

Fig. 4 : Comparison of *flrC* expression among *luxS* mutant strain of *Vibrio cholerae* MO10 lac⁻ & wild type. Transcription level of *flrC* was measured by Δ -galactosidase assay using *flrC promoter-lacZ* fusion transcriptional reporter construct

IV. Conclusion

It can be concluded that *luxS* gene may regulate the motility through the upregulation of trancription of *flrA* in *Vibrio cholerae* O139 Bengal strain MO10 lac⁻ but transcription of *flrB* and *flrC* were not influenced by *luxS* gene.

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