**Abstract**

**Thesis Title:** Adherence of *Vibrio cholerae* to human epithelial surfaces: Effect on virulence gene expression and global alterations in the bacterial proteome

*Vibrio cholerae*, a Gram-negative, noninvasive bacterium, colonizes the intestinal epithelium of human host, and produces several virulence factors including cholera toxin (CT) and a toxin-coregulated pilus (TCP) that greatly enhances colonization of the intestinal epithelium by the bacterium. Cholera toxin is a potent enterotoxin that causes the severe fluid loss characteristic of the disease cholera. Under laboratory conditions, the ToxR regulon is maximally expressed in *V. cholerae* grown at 30°C in LB medium, pH 6.6 and an osmolarity equivalent to 66 mM NaCl. Paradoxically, physico-chemical parameters thought to characterize the intestinal lumen, namely, a temperature of 37°C, an alkaline pH and high osmolarity, repress the expression of the ToxR regulon *in vitro*. Furthermore, bile a major constituent of the small intestine, represses the expression of virulence factors in *V. cholerae*. These observations suggest that as-yet unidentified signals encountered by *V. cholerae* in the intestine may overcome the repression of the ToxR regulon imposed by bile, alkaline pH and a temperature of 37°C. It is in this context that the present study has been undertaken to investigate the expression and mechanism of regulation of virulence gene expression in host cell-associated *V. cholerae*. In this study it has been demonstrated that adherence of *V. cholerae* to the intestinal epithelial cell line INT 407, strongly induces expression of the major virulence genes ctxAB and tcpA and the virulence regulatory gene toxT, and it could also overcome the repressive effect exerted by bile. No induction of toxR and tcpP, encoding transcriptional activators of toxT, was observed in the adhered bacteria. A sharp increase in expression of the vieA gene, encoding a cyclic di-GMP phosphodiesterase (PDE) was observed in INT 407 cell-associated *V. cholerae* immediately after infection. Furthermore, it has been shown that, the PDE activity of VieAEAL domain is essential and adequate for upregulation of toxT expression. Hence, induction of vieA and the subsequent decrease in c-di-GMP levels might be responsible for the induction of toxT observed in INT 407 cell-associated bacteria. This is the first report that host cell contact is a signal regulating c-di-GMP levels in bacteria through the induction of a c-di-GMP PDE. In this study, the c-di-GMP signalling networks of *V. cholerae* were significantly extended and revealed that this ubiquitous second messenger is a regulator of pathogenicity.

In addition, proteomic analysis has been done and a variety of proteins were identified as differentially expressed in the host cell-associated bacteria indicating that they serve the adaptive functions for the survival and pathogenicity in the host and the intestinal environment conditions.