Abstract

*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.