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Title of the Thesis: Characterization of *Vibrio cholerae* regulatory protein CytR in chitin utilization and pathogenesis.

Abstract

Vibrio cholerae is a Gram-negative facultative pathogen that resides in the aquatic environment either as a free-living bacterium or in association with phyto-, zooplanktons, molluscs, crustaceans, and arthropods. It infects humans through contaminated food and water and causes diarrhoeagenic cholera. In the aquatic environment, *V. cholerae* feeds on their exoskeletal chitinous covering. *V. cholerae* releases extracellular chitinases ChiA1 and ChiA2 to degrade the chitinous exoskeleton by breaking the β-1,4-glycosidic linkage of the chitin polymer releasing GlcNAc, which are sensed and internalized by *V. cholerae* as a source of the nutrient. The regulation of sensing and chitinase secretion is ChiS dependent, which is a periplasmic two-component sensor histidine kinase. *V. cholerae* also colonizes in the intestinal epithelium of the human gut, where the mucus layer provides a niche for colonization because it harbours attachment sites like glycans. Mucus glycans also act as a direct source of nutrients for the bacteria. *V. cholerae* chitinases act on mucus glycans to produce GlcNAc like residues which act as a nutrient source. Reports have also indicated that bacterial chitinases (ChiA2) are also involved in disease pathogenesis. *V. cholerae* ChiS regulates the expression of chitinases and it plays an important role in pathogenesis. There is another regulator of chitinases in *V. cholerae* known as CytR. Since chitinases play a role in pathogenesis, we speculate that CytR might also be involved in controlling virulence cascade, which is ultimately attributed to the pathogenicity of *V. cholerae* and causes disease. CytR is a 37 kDa cytoplasmic protein belonging to the LacI family of transcriptional regulators present in some γ-proteobacteria including *V. cholerae*. Its role in the transport and utilization of nucleosides has long been studied in *E. coli*. In *E. coli* CytR negatively regulates a small set of nucleoside scavenging and metabolism genes including *udp*, *cdd*, *ompK*, *cytR* itself via CRP-dependent anti-activation mechanism. *V. cholerae* CytR is a functionally diverse protein and global regulator. It represses multiple nucleoside catabolism and scavenging genes and positively regulates competence, a majority of natural transformation genes, bacterial killing by inducing type VI secretion system (T6SS) gene clusters, and co-regulates chitinase genes along with TfoX. Recently it has been discovered that among many other roles, CytR is involved in the regulation of *V. cholerae* extracellular chitinase ChiA1 and ChiA2. During pathogenesis, *V. cholerae* has to survive in the intestinal environment, penetrate through the mucus layer, adhere to the intestinal epithelium, form colonies to secrete cholera toxin (CT).

We demonstrated that CytR positively regulates activation of sensor histidine kinase ChiS in the mucinous environment, thus affecting the secretion of chitinases and degradation of mucin. CytR deletion resulted in no flagella synthesis (by downregulating *flrB*, *flrC* and all of class III flagellar synthesis genes), which leads to reduced motility and mucin penetration. Furthermore, CytR not only has a marked effect on the adhesion of the intestinal epithelial cells but also positively controls CT secretions and other virulence traits. CytR deletion resulted in the downregulation of virulence genes like, *toxT*, *tcpA*, *ctxB*, *toxR*. Our result suggests for the first time that CytR positively controls hexosaminidase activity in *V. cholerae* in the presence of mucin *in vitro* as well as in the intestinal environment. β-hexosaminidase activity is a measure of ChiS activation and its effect on the chitin utilization pathway. The activation of ChiS promotes the expression of downstream chitin utilization pathway components like periplasmic β-N-acetyl-glucosaminidase. The expression of ChiS is also CytR dependent as revealed by the qRT-PCR data. Thus, it indicates that CytR positively regulates both ChiS expression and its activity. Therefore, we speculate that CytR helps *V. cholerae* to break down the thick mucus layer with the help of chitinases in a ChiS-dependent manner.

Next, we aimed to study some inhibitory compounds which might act on CytR and exert anti-virulence properties. We have selected four different types of inhibitors after a thorough literature survey which include phytochemicals [Curcumin (CC), Carvacrol (CV) and Trans-cinnamaldehyde (TC)], sugars [N-acetyl D-glucosamine (GlcNAc), Xylose, Xylitol, Allose, and Mannitol], small molecules [Na-3-hydroxy Butyrate (3HB) and NH125] and Family-18 chitinase inhibitors [Pentoxifylline (PEN), Caffeine (CF), Dequalinium chloride (DQ) and Theophylline (TH)]. Although none of the inhibitors act on CytR few of them showed interesting results regarding the virulence properties of *V. cholerae*. Our data suggest that Carvacrol, Allose, Na-3-hydroxy butyrate, and Dequalinium chloride acts as potent inhibitors of CT production, adhesion, and motility which ultimately reduces the pathogenicity of *V. cholerae*. These anti-virulence drugs target bacterial virulence factors and, unlike classic antibiotics, are aimed at disarming pathogens like *V. cholerae*.

NSChatterjee 22-02-2022

नवेन्दु शेखर चटर्जी / Nabendu Sekhar Chatterjee
(वैज्ञानिक - एफ / Scientist F)
आई.सी.एम.आर राष्ट्रीय कॉलरा और आंत्र रोग संस्थान
ICMR National Institute of Cholera & Enteric Diseases
पी-३३, सी.आई.टी. रोड, स्कीम-१०एम, बेलियाघाटा
P-33, CIT Road, Scheme-XM, Beliaghata
कोलकाता-७०००१० / Kolkata-700010