Abstract

Vibrio cholerae is a frightening pathogen that uses diverse pathogenicity determinants to cause diarrheal disease called "cholera". Of these, cholera toxin (CT) and toxin co-regulated pilus (TCP) are considered as central virulence factors used by O1 and O139 serogroups and are implicated in several *V. cholerae* outbreaks. It was reported that some non-O1/non-O139 serogroup strains lacking CT and TCP are appear to cause sporadic outbreaks and extra intestinal infections. Genome sequencing of AM-19226 (non-O1/non-O139 serogroup strain, TCP^-/CT^-) identified a type three secretion system (T3SS) along with 12 bonafide effector proteins. AM-19226 utilize this T3SS to deliver effector proteins directly into host cells thereby casues cellular subversion. Till date, a few T3SS effector proteins (VopF, VopE and VopX) have been gained functional characterization. The function of remaining effectors in the context of *V. cholerae* pathogenicity is not available. In this work, we paid attention on functional characterization of two effector proteins such as VopK and VopE using a yeast model system.

VopK, one of the *V. cholerae* T3SS effector proteins, has been involved in the pathogenesis of non-O1/non-O139 serogroup strains. This protein causes strong toxicity when expressed in yeast model system. In silico investigation of VopK uncovered a predicted catalytic traid containing MCF1-SHE (SHxxxE) serine peptidase domain at the C-terminus region. Each amino acid of catalytic traid (SHE) was replaced with alanine and the resulting VopK variants ($VopK^{S314A}$, $VopK^{H353A}$, and $VopK^{E357A}$) showed variable toxicity in yeast model system. Interestingly, substitution of glutamate with alanine at 357 position causes complete loss in toxicity whereas replacement of $serine^{314}$ and $histidine^{353}$ with alanine exhibited partial loss in toxicity without affecting the stability of variants. In addition, alanine scanning mutagenesis of WRFNE (WxxxE like motif) identified an essential $tryptophan^{406}$ in VopK toxicity. Taken together, this study identified crucial amino acids of VopK activity using site directed mutagenesis and yeast model system.

VopE, a mitochondrial targeting T3SS effector protein of *V. cholerae*, known to modulates mitochondrial dynamics by inactivation of Miro GTPases. Such activity results in disruption of innate immune signaling. Herein, we observed a dose dependent VopE mediated yeast growth inhibition and this toxicity was further enhanced under various stressors. Surprisingly, a VopE mutant lacking predicted mitochondrial targeting sequence (MTS) also causes partial lethality in the yeast system. By using various yeast genetic tools and well established stressors, we have

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demonstrated that VopE and VopE$^{\Delta MTS}$ alters cell wall integrity (CWI-MAPK) signaling pathway and have revealed crucial amino acids and regions contributing to VopE mediated lethality. Moreover, expression of VopE$^{\Delta MTS}$ together with VopX partially suppresses the lethal activity of VopX in yeast system. Collectively, this study suggests that VopE and its derivative VopE$^{\Delta MTS}$ attenuates the activation of CWI-MAPK signaling pathway, and proven the value of yeast model system in effector protein characterization.