

CalR is required for the expression of T6SS2 and the adhesion of *Vibrio parahaemolyticus* to HeLa cells

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Abstract *Vibrio parahaemolyticus* expresses one major virulence determinant T6SS2, which is constituted into three putative operons, i.e., VPA1027-1024, VPA1043-1028, and VPA1044-1046. CalR, a LysR-type transcriptional regulator, was originally identified as a repressor of the swarming motility and T3SS1 gene expression. As shown in this study, CalR binds to the promoter-proximal region of each of the three operons to activate their transcription, and moreover, CalR activates the adhesion of *V. parahaemolyticus* to HeLa cells. In addition, competitive EMSAs demonstrated that CalR acts as an antagonist of H–NS in *V. parahaemolyticus*. Collectively, these studies confirmed a new physiological role for CalR in *V. parahaemolyticus*.

Keywords *Vibrio parahaemolyticus* · T6SS2 · CalR

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Introduction

Vibrio parahaemolyticus, the leading cause of seafood-associated diarrhea and gastroenteritis, is a Gram-negative bacterium that naturally inhabits estuarine and marine environments. Humans are commonly infected by *V. parahaemolyticus* due to the consumption of raw or undercooked seafood. The major virulence factors involved in the pathogenicity of *V. parahaemolyticus* include thermostable direct hemolysin (TDH), TDH-related hemolysin (TRH), type III secretion systems (T3SS1 and T3SS2), and type VI secretion systems (T6SS1 and T6SS2) (Raghunath 2014).

Bacterial T6SS is a novel needle-like protein injection machinery that is involved in virulence, symbiosis, inter-bacterial interactions, and antipathogenesis (Records 2011). The pandemic *V. parahaemolyticus* strain RIMD2210633 possesses two T6SS loci, named as T6SS1 and T6SS2, respectively (Makino et al. 2003). T6SS1 (VP1386-1414) harbors 29 consecutive genes forming 7 putative operons, while T6SS2 (VPA1024-1046) is constituted by 23 consecutive genes in 3 putative operons, i.e., VPA1027-1024, VPA1043-1028, and VPA1044-1046 (Ma et al. 2012). T6SS1 is a major contributor to antibacterial activity that can enhance environmental fitness of *V. parahaemolyticus* in marine environments, while T6SS2 is designed for the adhesion of *V. parahaemolyticus* to host cells (Salomon et al. 2013; Yu et al. 2012). Expression of both T6SS1 and T6SS2 is regulated by the quorum sensing (QS) system, the histone-like nucleoid structure DNA-binding protein (H–NS), and the environmental growth conditions (such as temperature and salinity) (Ma et al. 2012; Salomon et al. 2013; Wang et al. 2013; Sun et al. 2014).

LeuO is a LysR-type transcriptional regulator present in many Gram-negative bacteria (Hernandez-Lucas and Calva 2012). In *Salmonella*, LeuO is a global regulator that acts as