[SPI-5]

Expression of the *Vibrio vulnificus cadBA* Genes Required for Acid Tolerance is Dependent on a Transcriptional Regulator Protein, CadC

시작→

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The pathogenic marine bacterium *Vibrio vulnificus* is the causative agent of food-borne diseases, such as life-threatening septicemia and possibly gastroenteritis, in individuals with underlying predisposed conditions. Like many other food-borne pathogenic bacteria, *V. vulnificus* has to cope with ever-changing acidity in their growth environments to ensure developing illness. The *V. vulnificus cadBA* genes encode a lysine/cadaverine antiporter and a lysine decarboxylase, whose combined activity leads to the synthesis and excretion of cadaverine to counteract external acidification. Lysine decarboxylase activity of *V. vulnificus* was induced at a low pH (pH 5.8), and the induction of lysine decarboxylase was regulated at the level of transcription. A primer extension analysis revealed that *cadBA* genes are organized as a single transcriptional unit, and that the transcription of *cadBA* begins at a specific site, consisting of a putative promoter P*cad*. An open reading frame, *cadC*, consisting of 526 amino acids, was identified upstream of P*cad*. The production of lysine decarboxylase and the cellular level of *cadBA* transcript decreased in *cadC* mutant, which was constructed by allelic exchange. This decrease in the level of *cadBA* transcript in the *cadC* mutant appeared to be mediated by the reduced activity of P*cad*. These results establish that *cadBA* expression is directed by P*cad* in a pH-dependent manner, and activated by the gene product of *cadC*.

[SPI-6]

Molecular Analysis of the Regulatory Effect of SmcR and CRP on the Expression of Elastase in *Vibrio vulnificus*

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Among the putative virulence factors of *Vibrio vulnificus* is an elastase, the gene product of *vvpE*. It has been demonstrated that *vvpE* expression is differentially directed by two different types of promoters, PL and PS, and elevated by RpoS, CRP and a trans-acting regulatory protein SmcR. In this study, a primer extension analysis revealed that the activity of log-phase promoter, PL, was unaffected by a null mutation of *smcR*. The mutation of *smcR* reduced the activity of stationary-phase promoter, PS, indicating that the effect of SmcR on the expression of elastase is occurred through PS requiring RpoS. The mutation of *crp* also reduced, even less extent, the PS activity; however, the additional inactivation of CRP did not influence the PS activity in the *smcR* mutant. This indicated that the effect of CRP on the PS activity is mediated by SmcR. GST-pull down and gel-mobility shift analyses revealed that CRP interacted directly with SmcR, and facilitated binding of SmcR to the promoter. The binding sites for CRP and SmcR were mapped by deletion analysis of the *vvpE* promoter region and confirmed by an *in vitro* DNase I protection assay. Each binding site for CRP and SmcR was juxtapositioned and centered at the 214 and 184 base pairs upstream of the transcription start site of PS, respectively. The combined results demonstrate that the activation of *vvpE* expression by by CRP and SmcR is occurred in a growth-dependent manner through PS, and the regulatory proteins exerts their effects by directly binding to the promoter in the stationary phase.