Effect of VirF on the Promoter Activity of *yscW-virF* Operon in *Yersinia enterocolitica*

Thuan Nghi Vu[1,2] and Glenn Marvin Young[1]

[1] Department of Food Science and Technology, College of Agricultural and Environmental Sciences, University of California at Davis, Davis, CA, USA
E-mail: <thuannghivu@yahoo.com; gmyoung@ucdavis.edu>

Abstract

The Yerisinia enterocolitica *Ysc-Yop T3SS and its Yop effectors are essential for the bacteria to survive and overcome the host immune system. Expression of Ysc-Yop T3SS at the transcriptional level is thermo-controlled by the AraC transcriptional activator VirF, encoded by virF. The role of VirF in transcriptional regulation of* Y. entercolitica *Ysc-Yop T3SS genes has been intensively investigated but little in known about how the virF gene is controlled. This study was aimed at understanding how virF expression is controlled. The results showed that the virF, together with the yscW located upstream of virF are transcribed as an operon from the promoter P_{yscW}. We further assessed whether VirF might control transcription from P_{yscW} using a transcriptional fusion to a lacZ reporter. The analysis revealed that VirF does not have any influence to activity of promoter P_{yscW} at low (26^{o}C) or high (37^{o}C) temperature.*

Keywords: *Yop effectors, host immune system, transcriptional activator, T3SS gene, operon, lacZ reporter.*

1. Introduction

Yersinia enterocolitica, a gastro-intestinal foodborne pathogen, harbors the Ysc-Yop T3SS for its virulence. The whole Ysc-Yop T3SS including Ysc injectisome, secreted Yop proteins and their chaperones is encoded by the virulent plasmid pYV (Cornelis *et al*. 2002). In the absence of Ca^{2+} ions and at 37^{o}C, pathogenic *Y. enterocolitica* releases high amounts of Yop effectors that involved in pathogenesis (Lambert de Rouvroit *et al*. 1992).

The expression of Ysc-Yop T3SS is strongly thermo-regulated by transcriptional activator VirF (Lambert de Rouvroit *et al*. 1992). VirF, a 30.9 kDa protein, belongs to the AraC family of regulators and is encoded by the *vir*F gene that is localized just downstream of the *ysc*W gene on pYV virulent plasmid (Fig. 1) (Cornelis *et al*. 1989). In turn, the activation of *ysc* and and *yop* genes by VirF is controlled by YmoA.

At the temperature below 30^{o}C, YmoA stabilizes the DNA structure and thus inhibits VirF binding to the promoter regions of *ysc* and *yop* genes (Bleves and Cornelis 2000). After a shift to 37^{o}C, the change of DNA topology due to elevated temperature and the dislodgement of YmoA facilitates VirF binding to its recognized sites and activates the transcription of *yop* and *ysc* genes (Bleves and Cornelis 2000).

The role of VirF in transcriptional regulation of Ysc-Yop T3SS was well-studied by many authors. This study was initiated to identify mechanisms that affect transcriptional regulation of the *virF* gene. Data from our lab (unpublished), indicated that *virF* is cotranscribed with the upstream gene *yscW*. This *yscW-virF* forms an operon. This genetic organization resemble that of the *Pseudomonas aeruginosa exsCBA* operon, which control expression of T3SS genes in that bacterium. Interestingly, VirF shares 56% identity to *P. aeruginosa* transcriptional activator ExsA, which can autoregulate its *exsCBA* operon by binding to promoter regions of the *exsC* gene (Allaoui *et al*. 1995; Hovey and Frank 1995).

[2] School of Biotechnology, Assumption University, Bangkok, Thailand.