FELLOWSHIP SUMMARY REPORTS

Prof. Sang Ho CHoi

Title: Transcriptomic analysis of the *Vibrio vulnificus* pv. piscis exposed to fish mucus

Host institution: The University of Valencia, Spain

Host collaborator: Prof. Carmen Amaro

Dates of fellowship: July 3rd-Sept 30th, 2023

1. What were the objectives of the research project? Why is the research project important?

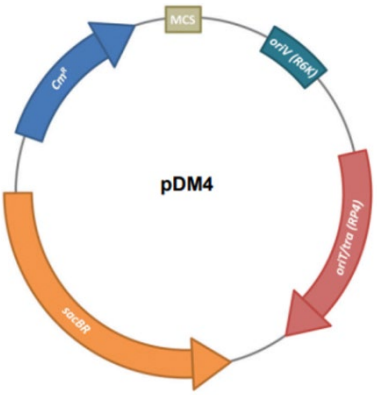
The fulminating pathogen *Vibrio vulnificus* pv. piscis is the causative agent for a range of diseases of marine animals including species of interest in aquaculture such as eels and shrimps. Our hypothesis is that upon being exposed to mucus of the marine animals, the pathogen differentially expresses genes involved in colonization, persistence, survival, and thereby cause disease. A whole-genome transcriptome profiles of the pathogen in the presence or absence of the fish mucus or purified mucin will be compared and thereby the virulence genes expressed specifically and required for the infection of the marine animals will be identified. Selected virulence genes will be cloned and further characterized at the molecular levels to expand current understanding of the *V. vulnificus* pathogenesis and thereby provide novel strategies to control the pathogen.

2. Were the objectives of the fellowship achieved?

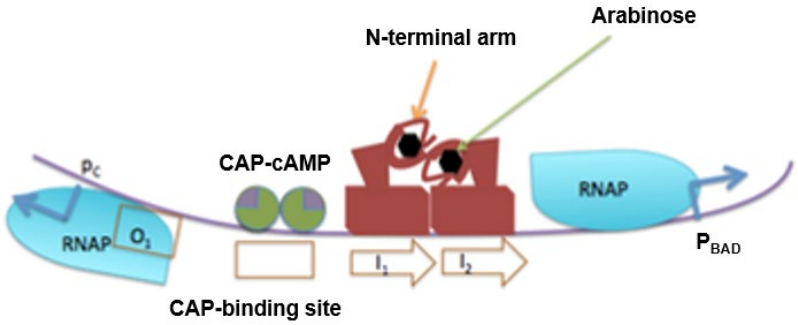
Yes, we have achieved the objectives. When we met together, the genes which are probably essential for the *V. vulnificus* pv. piscis to colonize, persist, survive, and thereby cause disease in marine animals had been identified and selected. To progress our collaboration further effectively, we discussed carefully the next step and then decided to rather characterize the selected genes together. To characterize the properties of the selected genes and their roles in the pathogenesis of the *V. vulnificus* strain, we decided to construct the mutants in which only the selected genes are specifically deleted and compared their pathogenic properties of the mutants with them of the isogenic parental wild type.

3. What were the major achievements of the fellowship? (up to three)

(a) We together set up the molecular biological system to construct a site-specific mutant of *V. vulnificus* pv. piscis in which a selected gene is inactivated. In detail, we decided to use the suicidal vector system pDM4 used previously in my group to construct mutants of *V. vulnificus* MO6-24/O. (Jang et al, J Biol Chem, 2016; Simon et al, Bio-Technol, 1983; Milton et al, J Bacteriol, 1996). Initially, the target gene was cloned, and then a significant portion of the target gene open reading frame (ORF) is deleted PCR *in vitro*. The deleted ORF fragment was cloned into pDM4. *E. coli* S17-1 λpir, containing pDM4 with the desired insert, was used as a conjugal donor to the parent strain to generate the deletion mutant. The mutant carrying the deletion in the target gene is now being selected and the deletion will be confirmed by using PCR.



(b) We together developed the molecular biological system to complement the mutants. In detail, respective ORF was amplified by PCR, and the amplified fragment was cloned into a broad host range vector pJK1113 under an arabinose-inducible promoter P_{BAD} (Lim et al, J Biol Chem, 2014; Guzman et al, J Bacteriol, 1995). The plasmids are now being transferred into the appropriate mutant by conjugation, and then the phenotypic complementation of the mutant will be examined.



(c) We together decided to measure the virulence and pathogenic potentials of the strains after the WT, isogenic mutants, and complemented strains of *V. vulnificus* pv. Piscis are constructed. If the virulence and pathogenic potentials are attenuated in the mutant of the selected gene, and further recovered in the complement strain, then we can postulate that the selected gene(s) is a novel and significant virulence gene.

4. Will there be any follow-up work?

I have come back to Korea. But a PhD student from Dr. Carmen Amaro's group will visit my laboratory next year to continue, extend, and complete our collaboration initiated by the support of OECD CRP fellowship this year.

If we further work together by exchanging the student, we can surely obtain important results. Then we will discuss together whether we publish the results or not. If we publish the data, we will acknowledge the support from the OECD CRP fellowship at any rate.

5. How might the results of your research project be important for helping develop regional, national or international agro-food, fisheries or forestry policies and, or practices, or be beneficial for society?

As mentioned previously, *Vibrio vulnificus* pv. piscis is the fulminating pathogen causing a range of diseases of marine animals including species essential in fisheries and as raw food sources such as eels and shrimps. If the selected genes are essential for the virulence and the pathogenesis of *V. vulnificus* pv. piscis,, they will be characterized at molecular levels. Then molecular characteristics of the selected genes can lead to the development of novel strategies to control the pathogen. For example, development of small molecules to inhibit expression of the selected genes can attenuate the virulence and pathogenesis of the *Vibrio* species. Attenuation of the virulence and pathogenesis of the pathogen could be beneficial to increase the production rate of the marine animals as important raw food sources.

6. How was this research relevant to:

The objectives of the CRP?

- *V. vulnificus* pv. piscis is the fulminating pathogen causing a range of diseases of marine animals including species including raw food sources such as eels and shrimps. Identification and characterization of the genes essential for pathogenesis of the pathogen can allow us to develop novel strategies to control the pathogens. The developed strategies can lead us to figure out the

ways to enhance the production of the raw seafood animals with enhanced security. The developed novel strategies to control the pathogens can further allow us decreased using antibiotics in the seafood cultures, leading to reduction of development of antimicrobial resistance strains of pathogenic bacteria.

The CRP research theme?
-My works in Spain has been related with Theme II, specifically food safety (antimicrobial resistance, too). *V. vulnificus* spp. are pathogens causing a range of diseases of marine animals including raw food sources such as eels and shrimps. *V. vulnificus* spp. are also causing disease of humane including fulminating and destructive septicemia. Therefore, if we successfully identify virulence genes essential for causing disease in marine animals and develop novel strategies to control the identified virulence genes of the pathogens, healthier marine animals, uninfected with the pathogens, can be produced. Production of the healthier and uninfected marine animals will allow us enhanced safety of the raw seafoods.

7. Satisfaction

Did your fellowship conform to your expectations?
-Yes, but I hope that the amount of support can be increased in the future.

Will the OECD Co-operative Research Programme fellowship increase directly or indirectly your career opportunities?
-Yes, before this collaboration, my researches have been focused only *V. vulnificus* pathogenic to human, but not to marine animals. Through this collaboration, I can expand my researches to the *V. vulnificus* pathogenic to marine animals, which are more essential for the pathogens to survive in nature before infecting human.

Did you encounter any practical problems?
-High living cost in Spain, exceeding the amount of OECD support.

Please suggest any improvements in the Fellowship Programme.
-Increase the amount your support.

8. Advertising the Co-operative Research Programme

How did you learn about the Co-operative Research Programme?
-From advertising brochure of Korean government grant agency

What would you suggest to make it more “visible”?
-In Korea, advertisement brochure of this program appears only once a year and is limited to the scientists working in the field of agricultures or fisheries. Hope, open the advertisement of more frequently and to the scientists in the diverse fields including basic sciences, too.

Are there any issues you would like to record?
-No. Thank you very much for your support.

9. References

1. Jang KK, Gil SY, Lim JG, Choi SH. Regulatory Characteristics of *Vibrio vulnificus gbpA* Gene Encoding a Mucin-binding Protein Essential for Pathogenesis. J Biol Chem. 2016; 291(11):5774–87. https://doi. org/10.1074/jbc.M115.685321 PMID: 26755724

2. Simon R, Priefer U, Puhler A. A Broad Host Range Mobilization System for Invivo Genetic-Engineering - Transposon Mutagenesis in Gram-Negative Bacteria. Bio-Technol. 1983;1(9):784-91. doi: 10.1038/nbt1183-784

3. Milton DL, OToole R, Horstedt P, WolfWatz H. Flagellin A is essential for the virulence of *Vibrio anguillarum*. J Bacteriol. 1996;178(5):1310-9. doi: DOI 10.1128/jb.178.5.1310-1319.1996. PMID: 8631707

4. Lim JG, Bang YJ, Choi SH. Characterization of the *Vibrio vulnificus* 1-Cys Peroxiredoxin Prx3 and Regulation of Its Expression by the Fe-S Cluster Regulator IscR in Response to Oxidative Stress and Iron Starvation. J Biol Chem. 2014;289(52):36263-74. doi: 10.1074/jbc.M114.611020. PMID: 25398878

5. Guzman LM, Belin D, Carson MJ, Beckwith J. Tight Regulation, Modulation, and High-Level Expression by Vectors Containing the Arabinose PBAD Promoter. J Bacteriol. 1995; 177(14):4121–30. https://doi. org/10.1128/jb.177.14.4121–4130.1995 PMID: 7608087