CHAPTER V

CONCLUSION

This thesis had reported the systematic investigation of the alternative method development for determination of *V.parahaemolyticus* based on its AHL production property. The first two experiments were optimization for AHL determination by colorimetry method, and validation of the methods. The next experiment was the study of influences of intrinsic and extrinsic factors associated with AHL production property of *V.parahaemolyticus*. In the last section, the evaluation and application of the developed method for *V.parahaemolyticus* determination were investigated.

The optimum AHL extraction condition and AHL determination using simple spectrophotometry were investigated. The protocol included using of 2 ml of chloroform to extract bacterial AHL, and the extracts was then dissolved in 800µl of D.I. water before adding each 1 ml of Reagent I and Reagent II allowing reaction of AHL color complex with ferric chloride to occur. The coloring complex was determined by spectrophotometry at maximum wavelenge of 520 nm (λ_{max}= 520). This AHL determination methodology was called procedure A. Another methodology, Procedure B, which was the determination of AHL existing in broth media without performing extraction step were also evaluated. The efficiency of both procedures were not significantly different in AHL determination. However, both methods were then subjected to validation.

The Procedure A and Procedure B were accurate, precise and selective for the AHL determination when they were validated following USFDA guideline. Minimal AHL concentration could be detected by both procedures was 30 µM. These methods could

detect AHL producing from *V.parahaemolyticus* cultured in NB containing minimal population approximately 6 logCFU/ml. Based on statistical analysis of each validation parameters, both procedures were potential to use as the AHL determination methods for further study. However, when considered the simplicity of the method, Procedure B was more advantageous since it was more rapid and less laborious. Therefore, Procedure B was selected to use for the further works.

According to the AHL production found in this study, intrinsic factor such as strains and extrinsic factor such as media compositions, temperature and growth phase of bacteria could not influence on quantities of AHL production of *V.parahaemolyticus*. High salt concentration (8% NaCl) was found to be a significant factor since it could induce *V.parahaemolyticus* to produce AHL in larger amount than the other conditions approximately 28%. Moreover, the result from the investigation of AHL production in each bacterial growth phase demonstrated that model of AHL signaling system in *V.parahaemolyticus* was more similar to up-regulation model than constitutive induction. This was reported for the first time in this study.

The influences of factors on qualitative AHL production were investigated using HPLC. According to HPLC chromatogram, *V.parahaemolyticus* always produced only 3-hydroxy-C4-HSL signal although it was cultured under different conditions. These results indicated that strains, cultivation periods, media, salt concentration, and incubation temperatures could not influence on the AHL type of *V.parahaemolyticus*. Therefore, 3-hydroxy-C4-HSL could be used as one of the *V.parahaemolyticus* identity. In addition, this study suggested that the produced AHL could be used as a finger print for identifying unknown bacteria culture due to its independence on strains and cultivation conditions as proposed in Figure 4.26 in chapter 4.

In the last section, procedure B with a modification was appiled. The selective medium 8%NaCl peptone water generally used for *V.parahaemolyticus* isolation were selected and evaluated. It was found that the 1%peptone containing 8%NaCl could be used for growing and inducing the AHL production of *V.parahaemolyticus*. The presenting of other *Vibrio* species in the culture system was also likely to induce the AHL production. Therefore, 8% NaCl peptone water was a potential enrichment selective media for growing *V.parahaemolyticus* before subjecting to AHL determination using procedure B. through this methodology, two logCFU/ml of *V.parahaemolyticus* in the cultures could be determined within 20 hours. A new strategy for isolation and identification of *V.parahaemolyticus* was proposed in Figure 4.25 (Chapter 4). This method could be an alternative for a rapid identification of *V.parahaemolyticus*.