CHAPTER VI

DISCUSSION

Seventy isolates of *Vibrio parahaemolyticus* were identified from 211 *Vibrio spp.* isolates using 23 selected biochemical tests. Of these 70 isolates, 32 isolates (41.71%) were found to have 4 atypical biochemical characteristics. Although the results are not corresponded to Bergey's manual of determinative bacteriology interpretation, yet, these 32 isolates are confirmed as *Vibrio parahemolyticus* using PCR method. Among 23 biochemical test used in this study, 17 tests are recommended as part of the routine set for *Vibrio* identification as described previously including methyl red test, arabinose fermentation test, 10 % NaCl concentration requirement and ornithine decarboxylase test, those which gave atypical characteristics. However, these four biochemical tests gave percentage of positive results, which are acceptable. The percentage of variabilities of positive interpretation are described by Farmer et al., 1992 as follow. Ornithine decarboxylation test gives 95% of positive result. Arabinose fementation test gives 80 % of positive result. MR test gives 80 % of positve result and the requirement of 10% NaCl gives 2 % of positive result. According to our results, biochemical conventional test could be useful for *Vibrio parahaemolyticus* identification,

nevertheless, a false positive in interpretation may occur by the percentage of variation.

The comparison of MIC_{90} and MIC_{50} values among the populations from different sources have shown that both MIC_{90} and MIC_{50} values of isolates from environmental source is higher than isolates obtained from clinical specimen (MIC_{90} = 3.3, 0.128 ; MIC_{50} = 1, 0.128), respectively. When comparing between isolates from different environmental sources, isolates from shrimp samples have shown higher value of MIC_{90} and MIC_{50} than isolates from sediment samples (MIC_{90} = 4, 2 ; MIC_{50} = 1.5, 0.5), respectively, and also be the group which have highest value of both MIC_{90} and MIC_{50} among all groups. The higher values of MIC_{90} in shrimp isolates indicated the more exposure of this group to ciprofloxacin since the isolates which exposed to ciprofloxacin will have MIC_{90} values more than isolates which are not exposed to ciprofloxacin. Besides, MIC_{90} values are related to resistant problem of quinolone usage. Fortunately, none clinical isolates in this study were found to have MIC_{90} values more than susceptible break point (MIC $\leq$ 1 μg/ml). This might be the result from behavior of people whom likely to eat cooked shrimp. Due to there is no specific interpretation criteria for ciprofloxacin susceptibility break point of *Vibrio parahaemolyticus*, hence the susceptible break point belonging to the closet Family, *Enterobacteraceae*, was adapted to use in this study.

Although biochemical test is considered to be a standard method in routine laboratory for bacterial identification, but with some obligations for instance time and sensitivity, the more rapid, sensitive and specific method such as polymerase chain reaction (PCR) has been adapted to use instead of

biochemical test to compensate those disadvantages. In this study, PCR method using *gyrB* primer as described by Venkateswaran et al., 1997 was applied to support biochemical identification of 21 isolates of *Vibrio parahaemolyticus* which have MIC $\leq$ 1 μg/ml and the outcomes have been satisfactory and successfully. The criteria for selection *gyrB* as *Vibrio parahaemiolyticus* detection tool are due to *gyrB* gene has conserved regions for the development of PCR primer and % homology of *gyrB* is less than 16s rRNA which would be useful in both screening and confirmation test as described by Yamamoto et al., 1995. However, there were some modifications of annealing temperature, adjusted from 58°C to 65°C, and $MgCl_2$ concentration, adjusted from 3 μl to 2 μl per reaction (100 μl).

Sequence analysis of QRDR regions reveals point mutations at codon 83 in *gyrA* and codon 85 in *parC*. The mutations in *gyr*A QRDR were found in all MIC $\leq$ 1 μg/ml isolates excepted SMV37 and SMV43, which 200-bp amplicon cannot be amplified after several modifications and repletions. An explanation of this finding could be an occurrence of a mutation or deletion at the primer complementary region. A mutation at codon 83 in *gyrA* QRDR resulted in amino acid changes from Serine (AGT) to Leucine (ATT) and also corresponded to Okuda's study in induced-ciprofloxacin resistant *Vibrio parahaemolyticus* with MIC = 0.78 μg/ml.

The mutations in *parC* QRDR were found in 20 MIC $\leq$ 1 μg/ml isolates excepted SMV 48 which has MIC = 1 μg/ml. A mutation at codon 85 in *parC* QRDR resulted in amino acid changes from Serine (TCT) to Phenylalanine (TTT). However, our findings are not corresponded to Okuda's

study since he found mutations in *parC* QRDR in a strain which have MIC value $\geq$ 50 μg/ml.

Based on these result, it is possible that there are the differences in the range of MIC values which leading to the occur of stepwise mutations in *gyrA* followed by *parC* of laboratory induced-ciprofloxacin resistant *Vibrio parahaemolyticus* as described by Okuda et al. and naturally ciprofloxacin resistant mutant of *Vibrio parahaemolyticus*. Since the range of MIC which can trigger the occurring of mutation in *parC* after the mutation in *gyrA* in a step, is 6.25-50 μg/ml, while in this study the trigger point may be MIC $\leq$ 1 μg/ml. Another explanation for this finding could be *parC* which may not play any role in increasing MIC value or ciprofloxacin resistance.Yet, the further study needs to be done in the isolates which have MIC $<$ 1 μg/ml.

Quinolones have been used in both human and animals for variety purposes. With increasingly uses of quinolones, the possibility of an emergence of resistant form of bacteria can occur, inevitably. For *Vibrio parahaemolyticus*, there is no clinical report about resistant situation so far, yet the study in laboratory induced resistant mutant of *Vibrio parahaemolyticus* by Okuda et al. has shown that clinical isolates of *Vibrio parahaemolyticus* can become resistant by selection, stepwisely. Based on this reason the use of quinolones should be monitored and controlled as much as possible to prevent the emergence of *Vibrio parahaemolyticus* in the environment.