Chapter 5 Future Work

As the biological function of bacterial *pepD* is less known, the gene knock-out study on *V. alginolyticus pepD* followed by series of biochemical or morphology analysis will give us more informations of its role in prokaryotes. At the same time, since PepD affects the bacterial biofilm formation, biofilm assay should be performed and compared with both *V. alginolyticus* wild-type and *pepD* knockout strain.

Since the 7 residues examined in this study were apparently involved in the catalysis of PepD, the more precisely effects of different single mutant on PepD activity should be analyzed through the enzyme kinetics study. The saturated mutagenesis on these positions was also considered to clarify their roles in the peptidase activity of PepD. Besides, according to the recent mutagenesis study on human aminoacylase-1 protein, which is also the member of peptidase family M20, several residues outside the catalytic domain were demonstrated to be involved in the substrate binding and catalysis (Lindner *et al.*, 2005). Therefore, several polar or aromatic residues outside the putative active site of PepD could also be investigated by site-directed mutagenesis.

With absence of the structure of neither peptidase family M20 nor similar peptidase sequence being solved, crystallization on *V. alginolyticus* PepD was recommended. Furthermore, the crystal structure of the wild-type and mutant proteins combinded with the mutagenesis analysis data could give an insight into the catalytic mechanism of bacterial aminoacylhistidine dipeptidase.