CHAPTER 4

CONCLUSIONS

Lactobacillus paracasei strain HL32, isolated from human fecae, produced a new bacteriocin demonstrating inhibitory activity towards *Porphyromonas gingivalis*. The antimicrobial substance was resistant to treatment with lipase but sensitive to trypsin and α-chymotrypsin. It was heat resistant (60 min at 100 °C), stable over a wide range of pH (3–8.5) and retained the bacteriocin activity after ultrafiltration and dialysis. It acted as bactericidal activity at 0.14 mM and could bind a specific receptor with the evidence that bateriocin formed pores on *P. gingivalis* cell wall that allowed K^+ efflux. The purified bacteriocin was identified as a 56 kDa peptide by SDS-PAGE. The N-terminal portion was not identical to any sequences described in protein database or research reports. The sequence data revealed that bacteriocin is a 156-amino-acid. Cytotoxicity potency affects red blood cell hemolysis rather than fibroblasts as well as periodontal ligament. Our study revealed that saliva derived from healthy and periodontal volunteers showed antagonistic effects in antibacterial activity of the bacteriocin against *P. gingivalis*.

For a future direction of this study, it should focus on the action of bacteriocin on outer membrane of *P. gingivalis*, the genetic determinants and molecular modeling of bacteriocin. To prove the specific mechanism, it is nice to investigate an

influence of the bacteriocin on the integrity of the outer membrane using TEM. The uptake and efflux of [^{3}H] glutamate and [^{14}C] proline on the intact cells should be monitored in comparison with artificial liposome and isolated outer membrane vesicles. Since the biological macromolecules derived from living sources as bacteriocin cannot be easily chemical synthesized and purified to high degree of purity. The genetic engineering or recombinant DNA technology will assist in bacteriocin production that meets pharmaceutical requirements. The processes can be roughly divided into 5 steps: identify the gene that encodes target protein, clone the gene, engineer an expression system for the repetitive bacteriocin, optimize the DNA sequence to enhance protein expression, and verify the molecular and functional characteristics of recombinant DNA responsible for DNA responsible for bacteriocin. In addition, the expression of recombinant protein under the control of strong promoters will give the possibility of obtaining very large quantities of proteins. High purity of recombinant bacteriocin is also suitable for biophysical studies, such as x-ray crystallography or nuclear magnetic resonance spectroscopy. In another way, when the full primary sequence is known, the predictions of the tertiary structure that determine the biological effectiveness of molecule can be done by the computer modeling. Both techniques have greatly enriched in understanding three-dimensional structures of protein. X-ray crystallography also provides the three-dimensional structures in atomic details, in which the scattered wave of x-rays depends only on the atomic arrangement in the crystal. While NMR gives us window into protein dynamics on a time scale of about 10^{-9}-10^{-6} seconds. The bacterioin is probably fewer that 150 residues is simple to analyze by NMR modeling. A 150-300 residues

protein is solvable by using suitable strategies of isotopic labeling (e.g., uniform double and triple labeling-with ^{2}H, ^{13}C, and ^{15}N or selective labeling of different amino acids). Alternative method by fragmentation of bacteriocin can be obtained and purified by stepwise enzymatic cleavage of the protein following by determinations of the NMR spectra of the fragments then allow certain residues to be assigned to particular region of the macromolecule. However, the chemical shift of a given residue in the fragment is not always identical with that in the intact molecule. By comparison, x-ray crystallography provides ever more detailed information on three-dimensional structure of protein.