

VII. SUMMARY

In the present study, it was revealed that three isolates of antimicrobial producing lactic acid bacteria possessed the potent activity against *P. gingivalis* W50. They were *L. paracasei* subsp. *paracasei*. The growth phase and generation time of each isolate were determined for culturing these bacteria and harvesting the large amounts of bacteriocin contained in the culture supernatant. The appropriate harvesting period is the early-stationary phase.

For the characterization of their antimicrobial compounds in the cell-free supernatant, it may be summarized that the bacteriocins containing in these supernatant were acidic, heat-stable at 80°C and proteases-sensitive proteins. Forty percentage of ammonium sulphate was the minimal concentration for optimal precipitation of protein which was carried out to perform as the protocol for bacteriocin extraction. These crude proteins could be observed in both anionic and cationic zones. All protein bands exhibited their molecular weight more than 30 kDa. The major clusters of proteins were located in the range of pI between 4 and 6.

In the protein purification, anion exchange column chromatography was performed for capturing the interested anionic proteins. The strong antimicrobial activity toward *P. gingivalis* could be detected in the anion pooled fraction 23-27, the partially purified bacteriocin of all isolates. Among all isolates, this bacteriocin produced by B85/4 isolate showed the strongest antimicrobial activity and highest bacteriocin activity recovered. Such bacteriocin exhibited the anionic manner with their molecular weights at 38 and 87 kDa. It may be suggested that the above bacteriocin is belong to class III bacteriocin. Its minimal inhibitory concentration against *P. gingivalis* W50 was 4.1 mg/ml. *In vitro* killing time at 99.9% inhibition toward *P. gingivalis* W50 was 6 hrs.

Further study, these proteins should be purified with gel filtration and characterized in the proteomic level.