CHAPTER 5

CONCLUSIONS

Two hundred and forty nine Lactobacillus spp. were isolated from vagina of healthy women. Sixteen isolates showed inhibition activity against Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 but none of them could inhibit Candida albicans NCPF3153. Among them, Lactobacillus sp. SK5, SK6, SK7 and SK8 with high inhibition activities were tested for sensitivity to antibiotics. They were all sensitive to ampicillin, bacitracin, cefoperazone, ceftazidime, cephalothin, chloramphenicol, erythromycin, penicillin G and tetracycline. These four isolates were resistance to kanamycin, ketoconazole, metronidazole, norfloxacin, nystatin, streptomycin and vancomycin. They were tested for hydrogen peroxide production ability. Lactobacillus sp. SK5 was better hydrogen peroxide producer compared with Lactobacillus sp. SK6 while Lactobacillus sp. SK7 and SK8 could not produce this substance. To compare adhesion ability with human vaginal epithelial HeLa cells, Lactobacillus sp. SK5 showed the highest adhesion activity follow by Lactobacillus sp. SK8, SK6 and SK7, respectively. Some bioadhesive polymers i.e., hydroxypropylmethylcellulose (HPMC-E50), sodiumcarboxymethylcellulose (Na-CMC) and polyvinylpyrrolidone (PVP) were used to study for adhesion enhancement of these bacteria to HeLa cells. From this result, PVP could increase adhesion of all bacteria and had better activity than HPMC-E50 and Na-CMC. Lactobacillus sp. SK5 with high hydrogen peroxide production and high adhesion ability was identified for species using 16 S rDNA. It was L. fermentum. Lactobacillus sp. SK 5 was later cultured in MRS broth and the cell free supernatant was dialysed in dialysis tube with molecular weight cut off 3,500. The remain substance showed inhibition activity against vaginal isolates of bacterial pathogens i.e., Pseudomonas aeruginosa, Staphylococcus epidermidis, Gardnerella vaginalis, E. coli and Neisseria gonorrhoeae. This high molecular weight substance was supposed to be bacteriocin.
Lactobacillus sp. SK5 was lyophilized with 8% w/v solution of skim milk, lactose and malt extract. Survival of the bacterial cells after lyophilization was highest in skim milk. Lyophilized Lactobacillus sp. SK5 in skim milk powder was prepared as hollow-type vaginal suppository using combination of different molecular weight PEGs as a suppository base. The suppository was evaluated for its properties. Each suppository contained $1.32 \times 10^8$ cfu of bacteria with average weigh 2.32 g, melting point 35-44 °C, disintegration time 8.5 minutes and bacteria were released from the suppository after 25 minutes. The properties of suppository are acceptable. From its promising properties such as high inhibition activity against vaginal bacterial pathogens, produce hydrogen peroxide and high molecular weight inhibitory substances, high adhesion ability to vaginal epithelial cells and can be formulated as hollow-type vaginal suppository, Lactobacillus sp. SK5 should be intensively studied in other aspects in order to use as probiotic against vaginal bacterial pathogens.