CHAPTER 6
In conclusion, four Lactic acid bacteria strains isolated from vagina of healthy women have \textit{in vitro} properties that make them potential candidates for probiotic applications. Among the strains, \textit{L. brevis} DT24 exhibited interesting probiotic properties such as excellent pH and bile tolerance, cell surface traits like hydrophobicity and aggregations, and suppression of Uropathogenic \textit{E. coli} make it ideal candidate for genetic modification and development of live microbicide for UTI.

The antimicrobial activity of lactic acid bacteria may be due to a number of factors, including decreased pH levels, competition for substrates, and production of substances with a bactericidal or bacteriostatic action, including bacteriocins. The antimicrobial components secreted by probiotic strains may help to avoid pathogen colonization of vagina. Again, these antimicrobial components may find applications as food preservatives and in clinical studies. This is the first report characterizing the bacteriocin component of \textit{L. brevis} DT24, \textit{L. plantarum} DT6 and \textit{E. hirae} DT98. Bacteriocin DT24 was efficiently working against various pathogens, that is, Uropathogenic \textit{E. coli}, \textit{Enterococcus faecium}, \textit{Enterococcus faecalis} and \textit{Staphylococcus aureus}. Bacteriocin DT 6 was efficiently working against \textit{Enterococcus faecium}, Uropathogenic \textit{Escherichia coli}, \textit{Lactobacillus rhamnosus}, \textit{Pseudomonas} spp., \textit{Staphylococcus aureus} and Bacteriocin DT98 was efficiently working against \textit{Staphylococcus aureus} and Uropathogenic \textit{E. coli}. All antimicrobial peptides were relatively heat resistant and also active over a broad range of pH 2–10. They have been partially purified by ammonium sulfate precipitation and gel filtration chromatography and checked on reverse-phase high-performance liquid chromatography. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of bacteriocin DT24, bacteriocin DT6 and bacteriocin DT98 were approximately 7-kDa, 8.5 kDa and 6-kDa respectively. All peptides were inactivated by proteolytic enzymes, trypsin and lipase but not when treated with catalase, a-amylase and pepsin. From the available datum, none of the bacteriocins isolated from probiotic and GRAS category lactic acid bacteria are active against uropathogenic \textit{E. coli}. This is the first report of characterizing the bacteriocin component of probiotic \textit{L. brevis} DT 24, \textit{L. plantarum} DT6 and \textit{E. hirae} DT98 isolated from the vagina of healthy Indian women. The bacteriocins DT24, DT6 and DT98 may have potential application in the prevention and treatment of various diseases.

Bacteriocin colicin E2 (\textit{Col}E2) and Human antimicrobial protein cathelicidin (\textit{LL37}) showed antimicrobial inhibition against Uropathogenic \textit{E. coli} \textit{in vitro}. These two proteins as a
conventional protein-based microbicide candidate, are expected to be costly to produce and relatively unstable at ambient temperatures, making it an unrealistic product, particularly in economically disadvantaged regions. In addition, protein-based microbicides may also require administration of relatively high concentrations due to the relatively short half-life and/or rapid clearance of these molecules. So, in present study we employ a live colonizing *Lactobacillus* from the protective vaginal microflora with potent probiotic and antimicrobial potential which has been genetically enhanced to deliver an anti-UPEC protein directly to the cervicovaginal mucosa, the major portal of bacteria entry in women.

In this study, the colicin E2 and Cathelicidin genes were cloned in probiotic *L. brevis* DT24 for synthesis of antimicrobial peptides like cathelicidin and colicin E2 individually. In this strategy observed colicinE2 and Cathelicidin proteins expression both cell associated as well as extracellular pathway confirmed by SDS-PAGE and antimicrobial activity. The resulted recombinant strains encode peptide colicin E2 and Cathelicidin showed enhanced antimicrobial potential by three and two fold then wild type *L. brevis* DT24. Most of the clinical *E. coli* isolates were inhibited by recombinant probiotics strains either by *L. brevis* (ColE2), *L. brevis* (LL37) and some of them also inhibited by both the strains. Engineered *Lactobacillus* showed maximum microbicide activity as compared to wild type strain of *L. brevis* DT24.

Given the potent anti-UPEC activity demonstrated by *Lactobacillus*-derived colicin E2 and cathelicidin and the high expression levels achieved by a vaginal *Lactobacillus* strain, the successful colonization of the vaginal mucosa by these bacteria should produce sufficient antimicrobials like colicin E2 and cathelicidin proteins in the proximity of bacteria to significantly decrease the number of UPEC and thus have a major impact on the frequency of urinary tract infections. The safety and immunogenicity profile of the isolates as well as recombinant strains were to be investigated for further usage. Large scale production with the long term stability of the transformant and protein need to be further investigated.