Abstract

Background: Urinary tract infection (UTI) is one of the most common bacterial infection in women and nosocomial infection in the developed world. One in four of women will develop a recurrence after the antibiotic use. If predisposing factors are not identified and removed, UTI can lead to more serious consequences, in particular kidney damage, which leading to renal failure. Uropathogenic Escherichia coli (UPEC) are the primary cause of UTI. During menstruation elevated protein concentration, increase in oxygen and carbon dioxide concentrations and with decrease in vaginal lactobacilli, which all together contribute to urinary tract infections. Lactobacillus species are a predominant member of the vaginal microflora and are critical in the prevention of a number of urogenital diseases. With the scope and impact of drug resistant UPEC widening, nontraditional management options are increasingly being explored. There is no licensed vaccine available yet for prevention of UTI in humans, likely due to the challenge of targeting a relatively heterogeneous group of pathogenic strains in a unique physiological niche. A promising strategy is the use of probiotic bacteria belonging to the vaginal microbiota as live microbicides for the topical production of UPEC inhibitor. Here we report on the development of a novel, live microbicide that employs a natural vaginal strain of Lactobacillus brevis engineered to deliver the potent UPEC inhibitor like cathelicidin or colicin E2.

Aim: The present study was designed to isolate potential probiotic lactic acid bacteria from vagina of healthy women having a potential anti-UPEC activity. The bacterium was further used for incorporation of anti-UPEC protein like cathelicidin and colicin E2 for development of live microbicide for the treatment of urinary tract infections.

Materials and Methods: The objective of the study was to screen the probiotic characterization lactic acid bacteria isolates from vagina of healthy women. Various physiological features of the candidate probiotic isolates were preliminarily investigated, including tolerance to simulated gastric juice and bile salts, antimicrobial activity against UPEC and other pathogens and mode of action, antibiotic susceptibility, and in vitro aggregation. From selected probiotics which showed highest bacterial inhibition was used for genetic modification with incorporation of cathelicidin and colicin E2 gene individually and functional characterization of the recombinant product was
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monitored such as antimicrobial activity against standard UPEC as well as against clinical UPEC isolates.

Results: Biochemical tests and 16S rRNA gene sequencing confirmed that isolated strains which showing higher antimicrobial potential against UPEC were *Lactobacillus brevis* DT24, *Lactobacillus plantarum* DT6, *Enterococcus faecium* DT2, *Enterococcus hirae* DT98 and *Enterococcus faecalis* DT48. Based on the results, *L. brevis* DT24, *E. hirae* DT98 and *L. plantarum* DT6 are ideal *in vitro* probiotic candidate and showed antimicrobial potential against UPEC via bacteriocin-like peptide production. However *L. brevis* DT24 showed higher inhibitory properties against wide variety pathogens i.e. Uropathogenic *E. coli, Enterococcus faecium, Enterococcus faecalis* and *Staphylococcus aureus* as well as showed potential probiotic properties such as acid and bile tolerance, cell surface traits like hydrophobicity, auto and co-aggregation ability and producing bacteriocin-like substances, too, which was used for further experiments. The colicin E2 and cathelicidin genes were incorporated in secretion vector pSLP111.3 (with xylA promoter, slpA signal sequence, and cell wall anchor protein) and electrotransformed in *L. brevis* DT24 individually. Extracellular secretions of both antimicrobial peptides were confirmed by SDS-PAGE. The antimicrobial activity of recombinant *L. brevis* (pSLP-ColE2) showed 3-fold and *L. brevis* (pSLP-LL37) was showing 2-fold increased zone of inhibition as compared to *L. brevis* (WT). Most of the clinical isolates from UTI infected human sample of *E. coli* were inhibited by recombinant probiotics strain either by *L. brevis* (ColE2), *L. brevis* (LL37) and some of them also inhibited by both the strains.

Conclusion: This study provided evidences clarifying the effectiveness of probiotic *Lactobacillus* expressing antimicrobial peptides like colicin E2 and cathelicidin against standard as well clinical UPEC. The establishment of this *Lactobacillus*-based secretion system, with potential broad applicability, represents a major step toward developing an inexpensive yet durable approach to topical microbicicides for the mitigation of UPEC and other mucosally transmitted pathogens. The safety and immunogenicity profile of the isolates as well as recombinant strains were to be investigated for further usage.

Key words: Urinary Tract Infections, Uropathogenic *E. coli*, Probiotic, *Lactobacillus*, Antimicrobial activity.