ABSTRACT

Probiotics, although not a new concept, has only recently begun to receive an increasing level of scientific interest as an alternative to antibiotics as well as prophylactics in humans. Probiotics are defined as “live microorganisms which when administered in adequate amounts confer health benefits to the host.” Probiotic bacteria have been credited with a number of beneficial effects within the host including: maintenance of homeostasis in the gut, production of antimicrobial compounds, control of blood cholesterol levels, the suppression of allergies, prevention of cancers of the colon and modulation of immune function.

Antimicrobial and anticancerous effects of a probiotic strain of *Lactobacillus fermentum* isolated from normal human colonic mucosa have been carried out here in a series of *in vitro* experiments.

The main objectives of the study were isolation and identification of Lactic acid bacteria (LAB) from human colonic mucosa, evaluation of the probiotic potential of the bacterial strain, study of the anti-microbial potential of the probiotic strain and demonstration of the anti–cancer activity of the bacterial strain by *in vitro* experiments.

LAB are among the dominant bacteria in the colon. Lactobacilli colonize the intestine and contribute to the appropriate defence against external antigens, pathogenic bacteria, or viruses. LAB have also proven to reduce risk of colon cancer, inflammatory bowel disease, as well as diarrheal illnesses. LAB exert their antibacterial activity through the production of lactic acid, 2-pyrrolidone 5-carboxylic acid, and other metabolites such as hydrogen peroxide, short-chain fatty acids, and bacteriocins such as nisin, reuterin and plantaricin. LAB produce a number of adhesion factors such as the mucus-binding protein, mucus adhesion promoting protein, elongation factor Tu, chaperone GroEL, surface associated proteins, and aggregation promoting factors. Several studies indicate that LAB prevent the attachment of pathogens and thereby reduce their replication and prevent infection.

In the current study, 74 viable strains of LAB were obtained from 9 normal colonic mucosal biopsy samples. The strain with the best acid tolerance as well as bile tolerance (82% and 98% respectively) – which is the primary probiotic criteria, was chosen and identified as *Lactobacillus fermentum* (*L. fermentum*).

The anti-microbial potential of the *L. fermentum* strain was studied by co cultivating it with pathogenic bacteria. *L. fermentum* was able to inhibit the growth of enteropathogens like *Escherichia. coli*, *Salmonella*. Paratyphi A, and *Shigella. sonnei,* Adhesion inhibitions of pathogens to cell lines were demonstrated *in vitro* using HT 29 and HCT 15 colon cancer cell lines. The non-covalently linked surface associated proteins (SAPs) of *L. fermentum* were responsible for the adhesion inhibition. SAPs were isolated and the effect was proved through adhesion assays.

*L. fermentum* was also able to inhibit the growth of nosocomial pathogens like *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Biofilm formation on medical devices is one of the major ways of colonization and infection by these pathogens. It was demonstrated that *L. fermentum* culture supernatant (CS) was able to inhibit
the biofilm formation of these pathogens. *L. fermentum* also inhibited the growth and biofilm formation of small colony variants (SCVs) of *S. aureus* and also clinical isolate of Methicillin resistant *S. aureus* (MRSA) and multi drug resistant (MDR) *P. aeruginosa*. The cytotoxicity caused by the toxins of pathogens on mammalian cells were shown to be decreased in the presence of CS of *L. fermentum*.

The anti cancer property of *L. fermentum* was demonstrated by MTT assay on colon cancer cell lines by treating the cells with whey of the milk fermented by *L. fermentum*. It was shown that the whey was toxic only to colon cancer cell lines HT 29, HCT 15 and COLO 205, while the normal cell lines INT 407 and IEC 6 were not affected. DNA damage caused as a part of cytotoxic cell death in HT 29 cells were evaluated by comet assay. Most of the HT 29 cells appeared comet shaped on alkaline single cell gel electrophoresis. INT 407 cells however remained unaffected. Induction of apoptosis of cancer cell lines also occurred on whey treatment. This was shown by Annexin V staining and RT PCR.

Probiotics is a relatively new concept in India. The commercial probiotic cultures currently being used in India are of foreign origin. Inherent differences in gut flora of Indian population are known to occur, hence it is imperative to carry out efficacy studies in Indian population prior to their use in India. Therefore, there is an urgent need for development of indigenous probiotic strains of Indian origin, and hence the present study.