Isolation and identification of indigenous lactic acid bacteria having probiotic potential

DETAILED FINAL REPORT OF UNIVERSITY GRANTS COMMISSION (UGC) MINOR RESEARCH PROJECT (File No.: 47-575/13(WRO))

2014-2016

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EXECUTIVE SUMMARY OF THE FINDINGS (IN 500 WORDS)

Following a thorough literature survey lactic acid bacteria were considered as candidates with probiotic properties. Lactic acid bacteria have been used for centuries in fermented and non-fermented foods mainly because of their ability to transform foods in terms of better taste and nutritive properties. These microbes are now widely being used as what are known as probiotics. Dairy was chosen as a convenient and very probable source of potentially probiotic lactic acid bacteria with an intention to isolate putative lactobacilli. Lactobacilli are generally regarded as safe (GRAS) to use in food products with little or no pathogenic potential.

Raw milk from cow/buffalo and curd samples were procured from various dairy outlets of the city. Organisms from the raw milk/curd samples were isolated on deMann Rogosa Sharpe (MRS) medium. The putative organisms showing typical colony characteristics and appearing as Gram positive rods were subjected to preliminary tests such as motility (using stab culture technique), catalase test and analysis of fermentation pattern viz. homo/heterofermentation. Nonmotile, catalase negative, homo/heterofermentative isolates were next analysed for biochemical profile using the HiCarbo kit (HiMedia) that tests for utilization of 35 carbohydrates viz. lactose, xylose, maltose, fructose, dextrose, galactose, raffinose, trehalose, melibiose, sucrose, L-arabinose, mannose, inulin, sodium gluconate, glycerol, salicin, dulcitol inositol, sorbitol, mannitol, adonitol, arabitol, erythritol, α-methyl D-glucoside, rhamnose, cellobiose, melezitose, α-methyl D-mannoside, xylitol, ONPG, esculin hydrolysis, D-arabinose, citrate, malonate and sorbose.

To exert their beneficial properties, probiotics must have robust survival properties in the gut. Studies have shown that very few organisms are indeed capable of this. The probiotic potential of the organisms was tested *in vitro* by using the ICMR-DBT guidelines for evaluation of probiotics in food.

Thus, probiotic characteristics of the isolates such as resistance to gastric acidity and bile acid resistance were next studied and only those isolates showing reasonably good survival (at least 50% for the harshest

conditions i.e. pH2.5 and bile acid concentration 1.5%), were further selected for identification at genus and species level. Identification of the organisms was done by 16S rRNA sequencing and sequence compared with data on the Genbank database of National Centre for Biotechnological Information (NCBI), USA. Most of the organisms were strains of *Lactobacillus fermentum* species.

There is experimental evidence that probiotic effects are strain specific. Functional and health promoting properties viz. antimicrobial activity, ability to reduce pathogen adhesion to enterocytes and bsh activity of the chosen isolates were next evaluated to select for the best organism showing most promising attributes. The antimicrobial activity was tested against common enteric pathogens like *Escherichia coli*, *Salmonella typhi* and *Proteus* spp. The ability of the chosen isolates to reduce pathogen adhesion was tested using Caco-2 cells and pathogens included *Escherichia coli*, *Salmonella typhi* and *Proteus spp*. The desired probiotic organism was selected based on a good antimicrobial spectrum and the ability to reduce adhesion of pathogens to the intestinal cells.

Thus the isolate Lc1 – identified as *Lactobacillus fermentum* was chosen and can be studied further to incorporate into diet as a probiotic organism.

Characteristics of Lc1 – *Lactobacillus fermentum*:

Acid tolerance (Survival %)		**Bile tolerance (Survival %)**		**Antimicrobial activity against**	**Ability to reduce pathogen adhesion**
pH 2.5	**pH 3.5**	**1.5%**	**0.3%**		
72	82.5	65	71.5	*E.coli, S.typhi, Proteus spp*	Very good