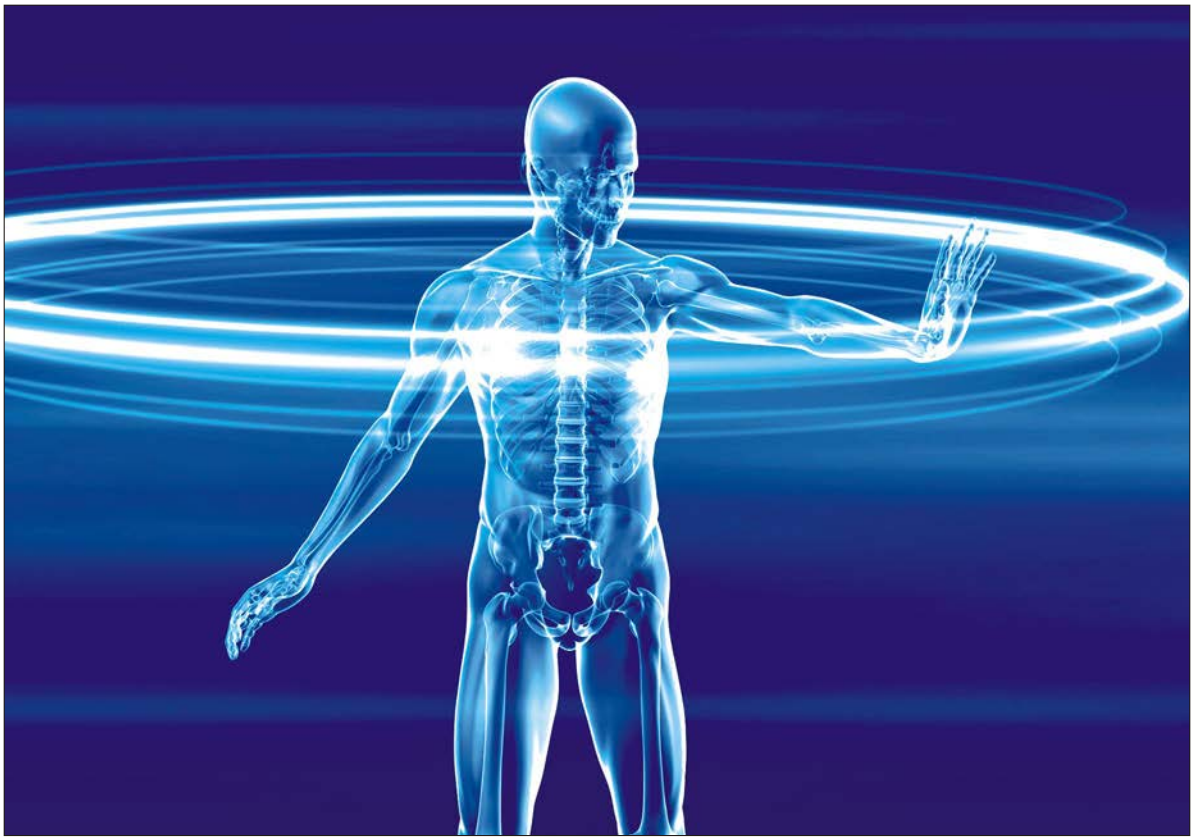

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Probiotic Action of Lactobacillus Isolated from the Milk Sample against Some Human Pathogens

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ABSTRACT

Probiotic efficiency of lactobacillus strains was isolated from milk sample and their antibacterial activities against human bacterial pathogens were screened in the present study. The milk samples were collected in sterile containers from different places of Tamil Nadu, India. The samples were analysed qualitatively and microbiologically. *Lactobacillus* was isolated from the milk samples and it was identified by biochemical tests. Methylene blue reduction test, which is colour sensitive to oxygen concentration is added to the milk. Resazurin test used to screen milk quality based either on the colour production. The total bacterial population count of milk sample was enumerated by Pour plate technique. MRS agar plates were prepared and a loopful of milk sample is taken. Quadrant streak procedure was carried out to isolate *Lactobacillus*. The isolated strains are identified and used to study their antagonistic efficiency against human bacterial pathogens isolated from different pathologic medium from patients diagnosed to have various wound infection at the laboratory Joys, Nagercoil, Kanyakumari (district) Tamilnadu. The probiotic action of *Lactobacillus* was studied against some human pathogens like *Eschericia coli, Staphylococcus aureus*, *Enterobacter aerogenes, Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* and it was observed that *Lactobacillus* showed good antagonistic effect against these pathogens.

Key words: *Lactobacillus*, probiotics, immunity, bacteriostatic and bactericidal, human bacterial pathogens

INTRODUCTION

Probiotic cultures have been associated historically with cultured of milks and dairy products, from which there is substantial evidence for positive effects on human health and general well-being (Klaenhammer, 2000; Reuter, 2001). Several *in vitro* and *in vivo* experiments on antagonism of different lactobacillus strains against *Helicobacter pylori* and *Clostridium difficile*, *Campylobacter jejuni*, *E. coli* were performed by Nowroozi *et al.* (2004). All tested human lactobacillus strains were able to inhibit the growth of all strains of anaerobic human gastrointestinal pathogens (Strus *et al*., 2001).

Milk is an excellent culture media for many kinds of microbes. Milk is normally sterile before it is drawn from the cow. It is contaminated by the microorganisms, which are introduced from the udder during handling. Raw milk from a healthy cow has *Streptococcus, Lactococcus* and other

lactic acid producing bacteria. The gastrointestinal tract of vertebrate animals is the most densely colonized region of the human body with approximately 10^{12} bacteria in the large intestine (Tannode, 1995). The ingested bacteria which are present in milk could have positive influence on the normal microflora of the intestinal tract (Nowroozi *et al.*, 2004). The ingestion of probiotic foods stimulate cytokinin production (Lemonier, 1996), decrease faecal mutagenecity (Salminena *et al.*, 1998a) and enhance lactose digestion (Sanders, 2000). He hypothesized that the lactobacilli were important for human health and promote the formation of yoghurt and other fermented foods which are good for health. Hence, the present study investigated probitic and antibacterial activity of lactobacillus strains isolated from milk samples.

MATERIALS AND METHODS

Collection of milk samples: Raw milk samples were collected duration of February-April, 2010 at different places (Nagercoil, Thuckalay, Martandam, Rajakkamangalam and kulasekharam) of Tamilnadu and maintained aseptic conditions. The samples were brought to the Departmrnt of Biotechnology, Prathyusha Institute of Technology and Management, Thiruvallur, Tamilnadu, India for qualitative tests and microbial analysis.

Standard qualitative analysis

Methylene blue reduction test: In this test, methylene blue, which is color sensitive to oxygen concentration, is added to the milk. The indicator is blue in color in the oxidized state and white in reduced condition. The speed of color disappearance of methylene blue, which is proportional to the number of bacteria present, is taken as an indication of the bacterial load (Gibbs, 1974).

Resazurin test: In this test the quality was judged based either on the color produced after a particular period of incubation or on the time required to reduce the dye to a given end point (Nixon and Lamb, 1945).

Enumeration of total bacterial population: The total bacterial population count of milk sample was enumerated by Pour plate technique. The test sample was mixed with known volume of sterilized distilled water to make serial dilutions. After serial dilution with precaution, 1 mL of aliquots of appropriate dilutions of the sample was pipette out into sterile Petri dishes and 15 to 20 mL of sterile nutrient agar medium were poured. The medium and the inoculums were thoroughly mixed using turntable and the medium was allowed to solidify. Duplicate plates were also maintained. The numbers of bacterial colonies were counted after 48 h of incubation. The bacterial populations were expressed as number of Colony Forming Units (CFU) per gram samples analyzed.

Isolation of *Lactobacillus* from the milk sample: MRS agar (de Man, Rogosa and Sharpe agar) plates were prepared and a loopful of milk sample is taken. Quadrant streak procedure was carried out to isolate *Lactobacillus* as per Aggarwal (2006) method.

Antagonistic effect of *Lactobacillus* against some human pathogens: This is done by using Agar plate disc diffusion method. Filter paper disc diffusion technique in agar was employed for determining antimicrobial activity. Whatman No.1 filter paper discs of 6 mm diameter, placed in dry Petri plates, were autoclaved. The test sample in measured quantities was dissolved in minimum amount of acetone. Sterile filter paper No.1 discs were loaded with the sample. The 20 μL of test sample loaded in disc for screen antagonistic effect against selected pathogens.

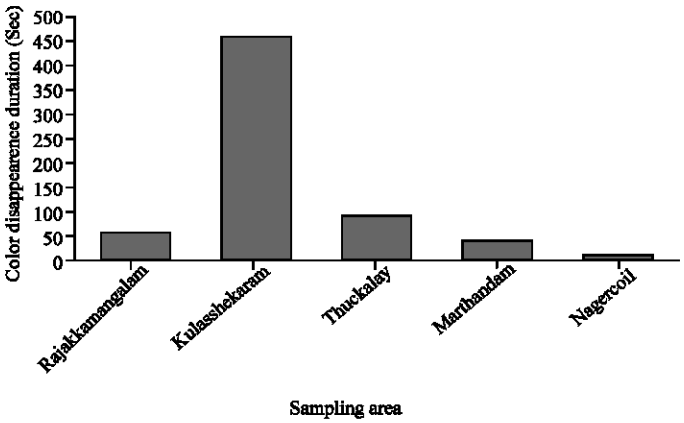

Fig. 1: Qualitative analysis of milk samples by MB reduction test

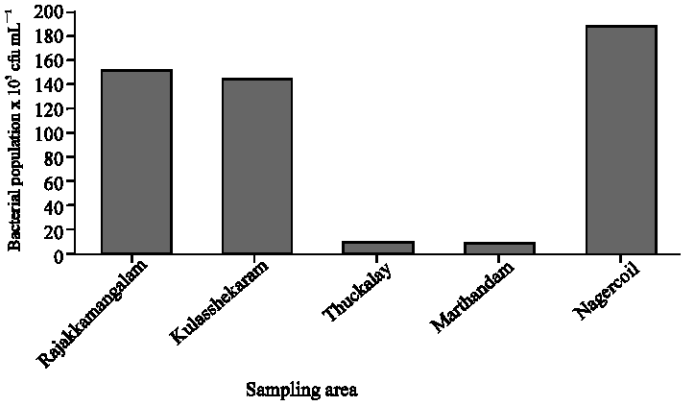

Fig. 2: Enumeration of total bacterial population in the milk samples collected from different locations

RESULTS AND DISCUSSION

Standard qualitative analysis of the milk samples from different places: Qualitative analysis of the milk sample was identified by dye reduction test. Quality of milk was analysed by organoleptic analysis. Good quality of milk is graded in 500 followed by poor qulaity graded upto 0 based on the quality. Sample collected from Nagercoil were very poor in Quality. The quality of milk sample collected from Rajakkamangalam and kulasekharam were better than the above. Sample collected from Thuckalay and Martandam was found to be poor (Fig. 1).

Enumeration of total bacterial population: Samples collected from Nagercoil contained 1.88×10^5 CFU mL^{-1}. Samples collected from Rajakkamangalam and kulasekharam contained less number of colonies i e., 1.53 and 1.47×10^8 mL^{-1}. Samples collected from Thuckalay and Martandam contained the 1.10 and 1.01×10^2 CFU mL^{-1} of microorganisms (Fig. 2).

Bacterial colonization of the intestine undergoes changes depending on age. From the present study we are anlysed efficiecncy of *Lactobacillus* in adult animals, it helps to improve immunity. But in childhood stage, it affects intestinal lamina it leads to suppress immunity. Bacterial colonization in early stage people is influenced by local immunity, bacterial fixation factors and the phenomenon of colonization resistance (Tournut, 1993). Also present study supported by

Table 1: Antagonistic effects of *Lactobacillus* against some human pathogens

Test organism	Zones (mm)
Salmonella typhimurium	16
Escherichia coli	7
Pseudomonas aeruginosa	17
Staphylococcus aureus	12
Streptococcus pyogenes	10
Shigella sp.	18
Klebsiella pneumonia	No zone formation

Jiang *et al.* (2001) bacterial strains from the neonatal period are replaced during the life by other bacterial strains characteristic of particular specimens and host. During the first days after birth qualitative and quantitative changes in the composition of the intestinal microflora are observed. At the time of weaning, lactic acid bacteria and coliforms are replaced by obligatory anaerobes (Berg, 1996).

Isolation of *Lactobacillus* from the milk sample by using selective media: *Lactobacillus* sp. was isolated from the milk samples by streak plate technique on MRS agar plates.

Identification of *Lactobacillus*: *Lactobacillus* was found to be Gram positive, catalase negative, motile, anaerobic organism. It was able to ferment carbohydrate to yield acid and gas. *Lactobacillus* cannot produce indole and it cannot utilize citrate but it can utilize lactose.

Antagonistic effect of *Lactobacillus* against some human pathogens: Using pathogens like *Escherichia coli, Shigella* sp., *Pseudomonas aeruginosa, Streptococcus pyogenes, Staphylococcus aureus,* and *Salmonella typhimurium* carried out the antagonism experiment. Growth inhibition of the test organism by *Lactobacillus* was also notices. *Lactobacillus* exhibited maximum inhibitory activity against *Shigella* by forming a zone of inhibition 18 mm. Next to this, *Lactobacillus* illustrated maximum inhibitory activity against *Pseudomonas aeruginosa* by forming a zone of inhibition 17 mm. Against *Salmonella typhimurium* it formed a zone of inhibition of 16 mm. Against *Staphylococcus aureus*, it formed a zone of inhibition of 12 mm. Least inhibitory activity was observed by *Lactobacillus* against *Eschericia coli* (7 mm). No inhibitory zone was formed by *Lactobacillus* against *Klebsiella pneumonia* (Table 1).

Pathogenic bacteria use different mechanisms to infect the gut. The two most important are adher-ence to the mucous membrane and the production of toxins. From the result *Lactobacillus* strains are help ful to eliminate predominant level of pahtogens (Hoepelman and Tuomanen, 1992). Several workers suggested not all pathogens are eliminated by mucous immune mechanisms because of their high binding affinity to surface glycoproteins, or glycolipids of the epithelial cells (Hook and Switalski, 1992; Svanborg, 1994; Forouhandeh *et al.*, 2010). The specific antibody-secreting lymphocytes appear in peripheral blood 2-4 days after antigen exposure, reach a maximum concentration after 6-8 days and persist in the blood for 2-3 weeks. Studies illustrate that these cells can reside in the gut. Homing receptors on lymphocytes, which interact with legends on endothelial cells, target the migration of lymphocytes into tissues (Salminen *et al.*, 1998b; Quiding-Jarbrink *et al.*, 1995). Antigen-specific systemic suppression after oral antigen introduction can be seen after 1-2 days and oral tolerance to systemic challenge becomes established within

5-7 days (Kantele *et al.*, 1996). Data suggest that interactions of lymphocytes with the intestinal epithelium are perhaps more important than what was realized previously (Strobel and Mowat, 1998; Ghafoor *et al.*, 2005).

Early exposure of the intestine to live micro-organisms and bacterial colonisation together with dietary antigens is very important for the development of the gut barrier (Helgeland *et al.*, 1996; Sudo *et al.*, 1997). Microflora boosts the barrier development through an increase in the duodenal Ig A plasmocyte population (Moreau *et al.*, 1978). It increases the number of enteroendocrine cells in the epithelium of the jejunum and colon, which enhances production of secretory IgA and mucus (Sharma and Schumacher, 1995).

Different mechanisms could influence the composition of the micro-organisms that colonise the digestive tract. The two important are: Antagonism among bacteria and local immunity (Osuntoki *et al.*, 2008). Disturbances in the ecological balance in the gut lead to the growth of harmful bacteria and to their possible translocation to internal organs, which induce disease. The intestinal microflora contributes to the processing of food antigens in the gut. Certain bacterial species isolated from the gastrointestinal microflora can liberate low-molecular-weight peptides, which trigger immune responses. Probiotic bacteria-derived proteases can degrade cow milk casein and thereby generate peptides with suppressive effects on the lymphocyte proliferation in healthy individuals (Kaila *et al.*, 1992; Murry *et al.*, 2004). To further characterize the immunomodulatory effect of probiotics, a study was designed to investigate whether caseins degraded by probiotic bacteria-derived enzymes could modulate the cytokine production with anti-CD3 antibody-induced, peripheral blood mononuclear cells in atopic infants with cow milk allergy (Perdigon *et al.*, 1998). Without hydrolyzation, casein increased the production of interleukin 4 in cultures from patients with atopic dermatitis, whereas *L. rhamnosus* GG-hydrolyzed casein reduced the production of interleukin 4. These results indicate that probiotics modify the structure of potentially harmful antigens and thereby alter the mode of their immunogenicity (Trachoo and Boudreaux, 2006).

CONCLUSION

Beneficially acting bacteria positively influence the immune system of the host. The protection of the mucous membranes is ensured through local immunity defense mechanisms. Their development is dependent on the direct contact of the host with antigens from the outside environment. The indigenous microflora joins in immune exclusion and protects the host from the adhesion of pathogens through competition for substrates and places of adhesion. These bacteria produce antibacterial substances and they stimulate the production of specific antibodies.

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