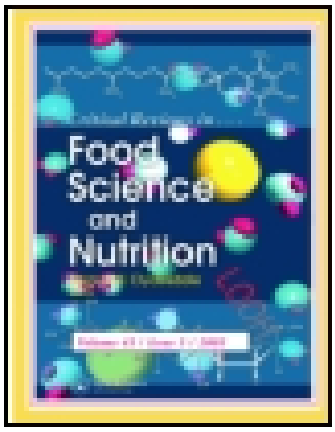

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Adhesion of Lactobacilli and Their Anti-infectivity Potential

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***Lactobacilli* adhesion and anti infectivity potential**

Adhesion of *Lactobacilli* and their anti infectivity potential

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Abstract

The probiotic potential of lactic acid bacteria primarily points towards colonizing ability of the *Lactobacilli* as the most important attribute for endowing all the known beneficial effects in a

host. *Lactobacillus* species exert health promoting function in gastrointestinal tract (GIT) through various mechanisms such as pathogen exclusion, maintenance of microbial balance, immunomodulation and other crucial functions. It have been seen that many surface layer proteins are involved in host adhesion and also play significant role in the modification of some signaling pathway within the host cells. Interaction between different bacterial cell surface proteins and host receptor have been imperative for a better understanding of the mechanism through which *Lactobacilli* exert their health promoting functions.

Keywords: *Lactobacilli*, colonization, surface layer proteins, health benefits

Introduction

Human large intestine harbors a complete microbiota and comprises of heterogeneous group of microorganisms (Fuller, 1989; Backhed et al., 2005). Bacterial density in the human colon is among the highest found in nature, approaching 10^{12} bacteria/gram faeces (Jason et al., 2005). In healthy individuals stomach and upper small intestine have relatively low number of microorganism as the host suppresses significant bacterial colonization in this part of intestine by a variety of mechanisms including rapid transit times, antimicrobial peptides, proteolytic enzymes, and hostile acidic pH and toxic bile concentration (Duary et al. 2012a; Kumar et al. 2011; Livingston et al., 2010). The lower small intestine is a transition zone between the sparely populated upper gastrointestinal tract and the heavily bacterially populated colon. In the lower ileum, the number of bacteria increases to the level of 10^6 to 10^7 organisms per milliliter of the content. However in the colon the bacterial concentration increases dramatically, reaching to a staggering figure of 10^{11} - 10^{12} organisms/ml of faeces (Fig. 1). This Gastrointestinal microbiota is considered as the first biological barrier against pathogenic bacteria (Yadav et al. 2013; Vanessa et al., 2006). The biological barrier constitutes several species of live microorganisms bestowing beneficial effects and acting deterrent to the pathogenic invasion, collectively termed as Probiotics. The definition of probiotics keeps on evolving pertaining to enormous increase in interest and research as these microorganisms have been scientifically shown to influence the composition and activity of the intestinal microbiota which reverberates to beneficial effects in the host (Salminen et al., 1996; Bouzaine et al., 2005). However, the widely acceptable definition of probiotics is ñLive microorganisms which when administered in adequate amounts confer a

health benefit on the hostò (FAO/WHO, 2009). Probiotics include Lactic acid bacteria, moulds and yeast; however, amongst the bacteria particularly belonging to lactic acid bacteria, *Lactobacilli* strains are considered as most potent probiotics along with *Bifidobacterium*. They are in vogue commercially for over 25 years as probiotic cultures because of their tremendous nutritional and therapeutic potentials. *Lactobacilli*, major members of Lactic acid bacteria (LAB), form a phylogenetically diverse group and are defined as Gram-positive, non-sporing, catalase-negative, anaerobic but aerotolerant, fastidious, acid tolerant and strictly fermentative bacteria that secrete lactic acid as the major end product of sugar fermentation (Axelsson, 1998; Mitsuoka, 1992). The ability to adhere to and colonize the intestinal or the urogenital tracts, even if transiently, are probably important factors that contribute to the survival of probiotic bacteria and thus help them to induce positive health effects on their host. For this reason, adhesive properties have been proposed by many authors as one of the criteria for the selection of new strains for probiotic use (Yadav et al. 2013; Duary et al 2011; Morelli, 2000; Dunne et al., 1999; Salminen & Von Wright, 1998; Goldin et al., 1992; Havenaar & Huis Veld, 1992). The interest in LAB as probiotics is primarily attributes to their traditional use in preservation of food grade and the fact that they are normally not associated with pathogenesis (GRAS status) and occurs naturally all through the GI tract.

It has been shown that probiotics can impart a number of health benefits (Parvez el al., 2006; Rastall et al., 2005), such as ability to synthesize and enhance the bioavailability of nutrients, modulate the immune system, reduce symptoms of lactose intolerance, decrease the prevalence of allergy, reduce the risk of certain cancers, infectious diseases and gut associated inflammatory

metabolic disorder such as ulceratic colitis, inflammatory bowel disease (IBD), crohnôs disease (CD), diabetes, cardiovascular diseases (CVD), rheumatoid arthritis (RA) etc.

Scope of the Review

Adhesion potential of *Lactobacilli* has been suggested as the pioneering factor influencing their transit time in the gut. Numerous studies have focused on the role of surface layer proteins in adhesion potential of putative probiotic *Lactobacilli*. Additionally, their structure, function, application and role in host-microbe interactions have also been discussed in detail. However, an extensive appraisal on role of different components in isolation and combined with a deliberation on in-vitro model culture system is needed to better understand the underlying mechanisms of bacterial adhesion to extracellular matrix (ECM) in the gut. The review discusses in details different surface layer proteins in general and collagen binding protein, a major surface layer protein, more specifically. The review also reflects on utilization of predominant model systems as a mean to simulate in-vivo gut conditions.

Lactic Acid Bacteria

There has traditionally been an agreement among scientists that LAB form a uniform bacterial group which in early times was referred to as ñmilk-souring organismsò (Orla-Jensen, 1919). They are relatively diverse group of bacteria related by a number of typical metabolic and physiological characteristics (Axelsson, 1998). Generally, the group consists of gram-positive bacteria, cocci or rods, which role are non-sporulating, non-aerobic and produce lactic acid as the major end product during fermentation of carbohydrates.

The introduction of modern molecular biology tool, in particular the comparison of ribosomal DNA sequences, has resulted in major revisions in LAB taxonomy and led to the

introduction of several new genera into this group. The current taxonomical schemes for the heterogeneous genera of *Lactobacillus* and *Pediococcus* are not in good agreement with the phylogenetic relationships revealed by 16S ribosomal DNA sequences and hence further changes are likely in the future (Chao et al., 2008; Axelsson, 1998; Stiles & Holzapfel, 1997).

The genus *Lactobacillus* includes essentially rod-shaped lactic acid bacteria and covers isolates with varying phenotypic, biochemical and physiological properties. Identification of many species of LAB including *Lactobacillus* is difficult by simple phenotypic criteria, such as biochemical tests. Other methods that are increasingly used now include determination of the % G+C of DNA, electrophoretic analysis of lactate dehydrogenase and soluble cellular proteins, ribosomal DNA sequencing, DNA-DNA hybridization of genomic DNA etc. (Forouhandeh et al., 2010; Tannock et al., 1999; Klein et al., 1998; Vandamme et al., 1996; Mitsuoka, 1992; Dicks and Vuuren, 1987). The genus *Bifidobacterium,* earlier included in LAB but later discovered evolutionarily distant is presently included in the *Actinomycetes* subdivision (Vandamme et al., 1996; Stackebrandt & Teuber, 1988; Woese, 1987).

LAB has complex nutritional requirements. In addition to carbohydrates, they need amino acids, peptides, fatty acids or fatty acid esters, salts, nucleic acid derivatives, and vitamins for growth (Sharpe, 1981). Two main carbohydrate fermentation pathways are utilized by LAB. Glycolysis via the Embden-Meyerhof pathway is used by the homofermentative LAB and 6-phosphogluconate/phosphoketolase pathway by heterofermentative LAB.

LAB as Constituents of the Intestinal Microflora

Major natural habitats of LAB are the gastrointestinal and urogenital tracts of humans and animals which provide stable conditions and a continuous supply of nutrients in the form of

ingested food and secretions of the host. *Enterococcus*, *Lactococcus, Lactobacillus* and *Bifidobacterium* are the dominating indigenous lactic microflora in the intestinal tracts of mammals and avian species. Amongst these *Lactobacilli* are the largest of the LAB genera and currently comprises more than 50 species. Growth of these bacteria is affected by several host mediated factors including acidity, bile salts, immunoglobulins, enzymes, exfoliated cells, mucins and tissue exudates as well as the peristaltic movement (Tannock, 1999; Holzapfel et al., 1998). To resist peristalsis bacteria either has to adhere to intestinal surfaces or multiply at a faster rate (Fuller, 1989). Since the peristaltic movement may be too rapid for significant bacterial multiplication (Drasar & Barrow, 1985), adherence to intestinal surfaces is an important factor contributing to successful colonization of bacteria in upper intestinal regions (O'Mahony et al., 2009; Tannock, 1992; Fuller, 1989).

In humans, the colon is the largest bacterial reservoir of the body. Movement of the contents is slow and travel through the colon takes 18-68 h (Mitsuoka, 1992), which allows the bacteria to multiply in the luminal contents. The bacterial communities in the colon are one of the most diverse in nature (O'Sullivan, 2000), with over 1000 described species (Masood et al., 2010; Duncan et al., 2007; Sears, 2005; Fooks et al., 1999; Moore & Holdeman, 1974). *Lactobacilli* are recovered in moderate numbers 10^4 -10^8/g wet weight (Lidbeck & Nord, 1993), while *bifidobacterium* average 10^{10} cells/g and constitute 5-10 % of the bacterial flora (Mitsuoka, 1992). However, the composition changes with decreasing number of *bifidobacterium* in adults (Reuter, 2001).

The Genus *Lactobacillus*

The genus *Lactobacillus* is extremely heterogeneous and includes a wide range of species with 32-53% G+C content of the chromosomal DNA arranged into their groups based on differences in sugar metabolism caused by the presence or absence of fructose-1, 6-diphosphate aldolase and phosphoketolase (Axelsson, 1998). Till date, 56 species of *Lactobacillus* have been identified. Commonly recovered *Lactobacillus* isolates from the human gastrointestinal tract include *L. acidophilus*, *L. salivarius, L. casei, L. plantarum, L. fermentum, L. brevis* and *L. reuteri* (Mikelsaar & Mandar, 1998). The frequency of *L. acidophilus* in the mammalian or avian gastrointestinal tracts and vagina has probably been overestimated since there has been a tendency to group all isolates of homofermentative *Lactobacilli* as *L. acidophilus* (Mitsuoka, 1992; Tannock, 1992; Johnson et al., 1980). According to the current identification criteria, *L. crispatus, L. gasseri* and *L. johnsonii* are the most common species of the *L. acidophilus* group in the human intestine (Song et al., 2000, 1999; Mitsuoka, 1992). Most of the intestinal strains formerly identified by fermentation patterns as *L. fermentum* are now classified as *L. reuteri* which is now regarded as the most prevalent heterofermentative *Lactobacillus* species in the intestinal tract of humans and other animals (Mitsuoka, 1992).

Accumulating evidences about the apparent ability of *Lactobacillus* as potent probiotic over a decade of intensive research has resulted in commercial use of different *Lactobacillus species* and strains which include *Lactobacillus* GG (*L. rhamnosus* or *L. casei* sub species *rhamnosus*), *L. acidophilus* NCFM, *L. casei*, *L. johnsonii* LA1, *L. ruteri*, *L. casei* strain shirota and *L. plantarum*.

***Lactobacilli* as Probiotics**

As already discussed probiotics are live microorganisms, which when consumed in adequate amounts confer beneficial health effects on the host (Guarner & Malagelada, 2003). If the complexity of the intestinal ecosystem inhabited by more than 1000 bacterial species and divided into several ecological niches is taken in consideration, it becomes clear that the selection of the bacteria to be used as a food additive or a biotherapeutic agent (Elmer et al., 1996) is not a simple task. It is also unlikely that each single strain belonging to one species possesses all of the characteristics that will make it a suitable probiotic. However there are some species among LAB which are reported most for their probiotic attributes. These include *Lactobacillus sp., Bifidobacterium sp*. and *Lactococcus lactis*. The strict anaerobic nature and also decreasing availability in adults of *bifidobacterium* reverberates in increased interest in *Lactobacillus* as potential prospective probiotics (Grover et al, 2012; Nagpal et al., 2012).

Several health promoting function have been found to be associated with probiotic *Lactobacilli*. In one of the essential studies, administration of *L. rhamnosus* GG was shown to enhance the production of IL-10, which acts as an anti- inflammatory mediator in atopic disease in children (Pessi et al., 2000; Heczko, 2007). In a more recent study an indigenous probiotic *L. plantarum* Lp91, under in vivo condition exhibited strong immunemodulatory properties in mouse colitis model (Duary et al. 2012a). Alleviation off diarrhea is a well-documented characteristic of some strains of probiotic *Lactobacilli* particularly *L. rhamnosus* GG whose ability to shorten the duration of acute rotavirus diarrhea has been well stabilized (De Roos & Katan, 2000).

Adhesion to the intestinal mucosa could confer a competitive advantage important for bacterial maintenance in the GIT and it is generally accepted that adhesion properties contributes

to the efficacy of probiotic strains (Yadav et al 2013; Servin & Coconnier, 2003; Bernet et al., 1994; Hudault et al., 1997). *L. rhamnosus* TB1 showed a good ability to adhere to intestinal epithelial cells of chicken with a good affinity to rectal tissues, ileum and jejunum (Bouzaine et al., 2005). Some studies have shown that *Lactobacilli* have the ability to colonize in the chicken crop. The indigenous *L. plantarum* strains exhibited strong adhesion potential showing higher percentage cell surface hydrophobicity (Yadav et al. 2013). However, in vivo experiments are needed to investigate and validate probiotic administration as a biological alternative to antibiotics as growth promoters for poultry and other food animals. Based on these finding, it is evident that to confer all these beneficial effects, a bacterium should realize all the probiotic attributes and to fully understand the importance of every factor involved, every single one requires a detailed attention and discussion.

The ability of microorganisms to survive in an acidic environment is important for both *in vivo* function and fermentation stability. Therefore, mechanisms contributing to the capability of a microorganism to tolerate acidic pH are essential to the production and functionality of a probiotic culture. Bacteria, used as probiotic adjuncts are commonly delivered in a food system and therefore begin their journey to the lower intestinal tract via the mouth. As such, probiotic bacteria should be resistant to the enzymes in the oral cavity e.g., lysozyme (Fuller, 1989) and should also have the ability to resist the digestion process in the stomach and the intestinal tract. Probiotic *L. plantarum* isolates (Lp9 and Lp91) under different in vitro pH conditions expressed atpD gene at pH 2.5 after 90 minutes incubation which demonstrated that expression of the 'atp' operon was chiefly instrumental in in vitro survival and tolerance of test cultures at acidic conditions encountered in the stomach (Duary et al 2010). Berrada et al. (1991) reported the

time from entrance to release from the stomach to be 90 minutes. However, further digestive processes have longer residence times hence there is a need for the bacteria to be resistant to the stressful conditions of the stomach (acidic pH) and upper intestine, which contains at toxic bile levels. The concentration of bile in the human gastrointestinal system is variable and is difficult to predict at any given moment. The ability to survive the action of bile salts is an absolute need of probiotic bacteria and is generally included among the criteria used to select potential probiotic strains. Bile acids are synthesized in the liver from cholesterol and are secreted from the gall bladder into the duodenum in the conjugated form (500-700 ml/d) (Kumar et al. 2011; Hoffman et al. 1983). These acids then undergo extensive chemical modifications (deconjugation, dehydroxylation, dehydrogenation and deglucuronidation) in the colon almost solely as a result of microbial activity (Hylemon & Glass, 1983; Shimada et al., 1969; Hill & Draser, 1968). Both conjugated and deconjugated bile acids exhibit antibacterial activity inhibiting the growth of *Escherichia coli* strains, Klebsiella *sp.* and *Enterococcus sp. in vitro* (Stewart et al. 1986). However, the deconjugated forms are more inhibitory and gram-positive bacteria are found to be more sensitive than gram-negative bacteria (Floch et al., *1972*). Chou & Weimer (1999) screened *L. acidophilus* strains on the basis of acid and bile tolerance. These isolates were capable of rapid growth in MRS at pH 3.5 containing 0.2% mixed bile salts. No loss of viability was detected over 5 h of exposure to pH 3.0 indicating a naturally high level of acid resistance in *L. acidophilus* to hydrochloric acid (Azcarate-Peril et al., 2005). In a similar study carried out by Kaushik et al., (2009) *L. plantarum* 9 isolate when subjected to acidic pH (1.5 and 2.0) and bile salt concentration (1.5% and 2%) showed marginal decrease in the log (cfu/ml) value indicating efficient acid and bile tolerance in stomach and intestine, respectively.

It is only after traveling through the harsh environment offered in the stomach and small intestine does the organism can colonize in the epithelium of the lower intestinal tract (Conway et al., 1987). Cell surface hydrophobicity is one of the factors that may contribute to the adhesion of bacterial cells to host tissues. It may play an important role in the interaction of probiotic *Lactobacilli* and other lactic acid bacteria with the epithelial cells of the gastrointestinal tract. The adherence of these organisms to mucosal structures is generally believed to facilitate colonization and persistence of probiotic strains in the normal intestinal population (Geertsema et al. 1993). Kaushik et al. (2009) investigated the percent cell surface hydrophobicity of *L. plantarum* 9 against hydrocarbon (n-hexadecane and xylene) along with standard probiotic strains like *L. johnsonii* LA1 and *L. acidophilus* LA7. The hydrophobic values obtained for indigenous probiotic *L. plantarum* in the presence of n-hexadecane and xylene were almost similar (37.1ï 37.7%) while for *L. johnsonii* LA1 and *L. acidophilus* LA7, the observed values were 47% and 57ï 58%, respectively (Yadav et al. 2013; Kaushik et al. 2009). In a related study conducted by Ingegerd et al. *(*1996) *L. plantarum* strains belonging to different subgroups and five *E. coli* strains with mannose-specific adhesions were tested for their ability to adhere to human colonic cell line HT-29 cells. They reported mannose-sensitive adherence of the bacteria to human colonic cell line HT-29 cells, mannose sensitive agglutination of *S. cerevisiae* and erythrocytes from different species, and direct binding of the bacteria to D-mannose immobilized on agarose beads.

Kotzamanidis et al. (2010) reported that *Lactobacilli* strains DC421, 2035 and 2012 possessed certain cell surface traits such as hydrophobicity, autoaggregation and high adhesive capacity suggesting potential immunomodulatory activity. They observed cell surface

hydrophobicity to be an important component of a complex mechanism that enables a microorganism to interact with the host gut to exert its immunoregulatory activity. In a different study (Saeed & Heczko, 2007) *Lactobacilli* were isolated from mouth, stomach and intestine of healthy subjects and were examined for cell surface characteristics such as autoaggregation and adherence to KB cell line. It was found that all the *lactobacillus* strains showed basic surface character and those with high hydrophobicity scores and auto-aggregation were highly adherent to KB cell line. Taheri et al. (2009) reported that *L. johnsonii* LT171 and *L. crispatus* LT116 showed very high surface hydrophobicity (85.21 and 92.14% respectively) in physiological saline water against toluene inferring that the strong association of cell surface hydrophobicity and aggregation made them more suitable instead of the examination of adhesion ability to the mucus.

Nelly et al. (2004) reported that the bacterial cell surface hydrophobicity was increased when they enter from logarithm growth phase to stationary phase. In the case, when the whole broth was used as inoculum instead of washed cells, cell hydrophobicity did not change significantly during growth. The study concluded that cell surfaces of *Lactobacilli* may adapt their surface hydrophobicity in response to environmental changes, like pH or ionic strength. These factors play important role in adherence of a *lactobacillus* strain to the gut epithelium which is a prerequisite for its colonization enabling it to exhibit its functional attributes such as antimicrobial activity, immunomodulatory effects, and antioxidant activity.

Another important functional attribute of LAB is their ability to produce antimicrobial compounds. Bacterial antagonism has been recognized for over a century but in recent years this phenomenon has received more scientific attention, particularly in the use of various strains of

lactic acid bacteria. Several metabolic compounds produced by lactic acid bacteria (including organic acids, fatty acids, hydrogen peroxide, and diacetyl) have antimicrobial effects (Tuomola et al., 1999). However bacteriocins or proteinaceous substances with specific inhibitory activity against closely related species are also responsible for antimicrobial activity. Of these, nisin which is produced by some *Lactococcus lactis* strains is at present the only purified bacteriocin approved with GRAS status for use in products intended for human consumption (Jack et al. 1995; Dodd & Gasson, 1994).

The ability to cope with reactive oxygen radical is key to another functional attribute of probiotic *Lactobacilli* i.e. antioxidant activity. The interest in reactive oxygen species (ROS) in biology and medicine is evident because of their strong relationship with phenomena such as aging and disease processes (Cao et al., 2008). The concept of ROS comprises not only oxygen centered radicals such as O_2 and OH, but also non-radical derivatives of oxygen such as H_2O_2 and hypochlorous acid. It is well known that free radicals and other ROS are continuously being produced in living organisms. As a consequence, defense mechanisms have evolved to deactivate these free radicals and repair the damage caused by their reactivity (Halliwell & Chirico, 1993). However, these systems are not always sufficiently active to disarm the totality of metabolically produced or exogenous free radicals. The interest in antioxidants is growing because of their antimicrobial activity. Despite advanced food production and preservation techniques, the spoilage and poisoning of foods by microorganisms is still the problem. The consumersô acceptance for preservatives with chemical origin is decreasing. Therefore, the producers are looking for natural compounds which can be an alternative and supplemented to food products to prolong their shelf-life and microbial safety (Aleksandra et al., 2008). Intracellular cell free

extract from *Lactobacillus* species has been shown to be a suitable substitute for vitamin E deficiency and thereby reducing the oxidative stress in rats (Kaizu et al., 1993). Lin & Yen, (1999a) reported antioxidative activity of yoghurt microorganisms such as *Streptococcus thermophilus* and *L. delbrueckii* subspecies *bulgaricus* by inhibition of linoleic acid peroxidation. Most of the lactic acid bacteria have natural defense systems to cope with oxygen radicals. According to Stecchini et al. (2001), the most common systems are superoxide dismutase and high internal concentrations of Mn^{2+}. Knauf et al., (1992), reported that some species of *Lactobacilli* produced a heme dependent catalase, which can degrade H_2O_2 at a very high rate, blocking the formation of peroxyl radicals. The ability of lactic acid bacteria to create low oxidation-reduction potential needed for their optimum growth probably is related to some of these systems. Free radical scavenger properties of starter lactic cultures would be useful in the food manufacturing industry. They could beneficially affect the consumer by providing another dietary source of antioxidants (Tuomola et al., 1999) or by providing probiotic bacteria with the potential of producing antioxidants during growth in the intestinal tract. Several species of *Lactobacilli* and *bifidobacteria* have been reported to produce antioxidative activity (Kaushal & Kanshal, 2011; Kullisar et al., 2002; Korpela et al., 1997; Zaizu et al., 1993).

Lactobacillus casei, *Lactobacillus acidophilus* and *Lactococcus lactis* and milk fermented with these strains have been studied extensively for antioxidant and cholesterol assimilation activities in vitro and in vivo, in addition to the effect on total *Lactobacilli*, *Lactococci* and coliform counts into the gut of mice fed with diets supplemented by fermented milk. All the three selected strains exhibited potential 2,2-diphenyl-1-picrylhydrazyl, malonaldialdehyde and hydrogen peroxide radical scavenging abilities as well as linoleic acid

peroxidation inhibition activity (Lin & Yen 1999b). These activities were highest in *Lactobacillus casei* as followed by *L. acidophilus* and *L. lactis*. In addition, these bacterial cultures also exhibited good in vitro cholesterol assimilation potential. Oral administration of milk fermented with selected LAB strains to mice, slightly decreased blood cholesterol, increased colonization of total *Lactobacilli* and *Lactococci*, and decreased coliforms in the intestinal tissues as well as faecal samples. These results indicate that selected LAB strains had demonstrated good antioxidant, hypocholesterolemic and coliform removal activities. It may suggest that, a novel functional food can be obtained by supplementation of selected LAB in milk, which may have various health beneficial properties such as antioxidant and hypocholesterolemic activities (Jain et al., 2009). In a recent study carried out by Sun et al. (2010) nine *Lactobacillus* strains were studied for the inhibition of lipid peroxide formation. *L. paracasei* Fn032, *L. rhamnosus* GG (LGG) and *Lactobacillus* species Fn001 exhibited moderate to strong antioxidative property. *L. rhamnosus* GG and *L. paracasei* Fn032 significantly decreased hydroxyl radicals ($P < 0.01$) in colonic fermentation model, in which considerable hydroxyl radicals occurred spontaneously. Addition of ferrous ion induced the production of hydroxyl radicals, which could be significantly inhibited by *L. rhamnosus* GG, *L. paracasei* Fn032 ($P < 0.01$) and Fn001 ($P < 0.05$). Ferrous ion significantly induced the growth of *Enterococcus* and *Escherichia coli*, which could be inhibited by all the three *Lactobacillus* strains.

The limited review above apparently shows that to be a probiotic, each factor of the criteria listed is equally important. However, adherence of probiotics to intestinal epithelial cells

and the ensuing temporary colonization of the gut are probably the most crucial factors for efficient donation of beneficial health effects (Hudault et al., 1997; Bernet et al., 1994).

Adhesive Properties of Probiotic *Lactobacilli* in the Gut

The importance of bacterial adhesion in infectious diseases is well established, and several molecular adhesion mechanisms of bacterial pathogens are already known **(**Avadhanula et al., 2006; Patti et al., 1994; Hultgren et al., 1991). The intestinal microflora in adults is considered stable and prevents colonization by exogenous bacteria, a phenomenon referred to as ñcolonization resistanceò (Vidal et al. 2010; Payne et al. 2003; Van der Waaij et al. 1971) or ñcompetitive exclusionò (Ramiah et al. 2008; Bech-Larsen & Scholderer, 2007). It involves several mechanisms such as occupation of available niches, secretion of growth-inhibitory factors (organic acids, hydrogen peroxide and bacteriocins) and non-specific activation of the immune system (Koenen et al. 2004). There are limited evidences which indicate the role of microbial adhesion on the mucosal epithelial cells as a colonization factor for their extended transit in the gut. Lactic acid bacteria have been shown to enhance macrophage, lymphocyte activation (Aattour et al., 2002; Marin et al., 1998), antibody production (Park et al., 2002), and natural killer-cell function (Haller et al., 2000). The ability of some *Lactobacilli* to competitively exclude pathogens from adhesion sites in the intestinal mucosa represents another means of defense against pathogens provided by probiotic microorganisms (Asahara et al., 2010; Ouwehand et al., 2001). One of the health-promoting effects of *Lactobacillus* is the prevention of microbial infections in the gastrointestinal and urogenital tracts. Possible mechanisms include immune modulation of the host and strengthening of the gut mucosal barrier against pathogens (Guarner, 2007; Holzapfel et al., 1998; Kasper, 1998). One of the significant properties of the

immune system is the ability to discriminate between pathogenic and non-pathogenic bacteria within this microflora. While many pathogens possess mechanisms to evade and defeat the immune system, some bacteria can positively modulate the immune system to successfully avoid and fight pathogens. Certain bacteria, including some probiotic *Lactobacilli* are known for their therapeutic efficacy in anti tumor and anti allergy immunotherapy and also for stimulation of a protective immune response to enhance resistance to microbial pathogens. Healthy immune function both systemic and local can be influenced by signals provided by Lactic acid bacteria including *Lactobacilli* in the intestinal tract (Cukrowska et al. 2010; Deepika & Charalampopoulos, 2010).

Adhesion to Extracellular Matrix

As already discussed adhesion factors are generally considered to play an important role in hostï microbe interactions and in pathogenic bacteria they have been shown to play a key role in virulence (Yadav et al. 2013; Hammerschmidt et al., 2008; Navarre & Schneewind, 1999). Analogously in probiotic bacteria, they are expected to play a role in persistence and competitive exclusion of pathogens or other health-stimulatory interactions (Marco et al., 2006). The extracellular matrix (ECM) is a relatively stable structure that underlies epithelia and surrounds connective tissue cells. ECM is involved in cellular development and function, growth and differentiation, cell adhesion as well as migration. The main components of ECM belong to four major classes of molecules the collagens, proteoglycans, structural glycoproteins (laminin, fibronectin, vitronectin, entactin) and elastin (De Leeuw et al. 2006; Styriak et al. 2003; Lorca et al. 2002). Basement membranes (BM) are a class of specialized extracellular matrices that appear as amorphous sheet-like structures between a cell layer and a thick collagenous stroma,

e.g. between the intestinal epithelium and the underlying connective tissue. Collagen and laminin are the major components of BM which interact to form a network structure. Adhesion to extracellular matrix proteins is expressed by several pathogenic bacteria and has been suggested to contribute to their invasiveness (Tallon et al., 2006; Westerlund & Korhonen, 1993). Among LAB strains, adhesiveness to ECM proteins has also been reported. Aleljung et al., (1994) demonstrated that binding to solubilized collagen is frequently expressed in *Lactobacillus* strains of different origins. In this study 75% of the LAB isolates were found to bind solubilized type I collagen. *Lactobacilli* isolated from dental caries lesions showed similar levels of binding to solubilized type I collagen (McGrady et al., 1995).

Major ECM protein, fibronectin, was expressed by 17% of human vaginal *Lactobacillus* isolates (Harty et al. 1994). Nagy et al., (1992) suggested a connection between the binding of ECM proteins by oral *Lactobacilli* and their ability to cause infective endocarditis (IE) in humans. Soluble fibronectin in saliva might coat the bacterial cells which invade into the bloodstream through damaged tissue sites and fibronectin could form a bridge between bacteria and the damaged endothelium of the heart valve. Harty et al. (1993) also reported efficient binding to immobilized collagen types I and V by *Lactobacilli* associated with infective endocarditis (IE). Type V collagen in particular has been demonstrated at sites of endothelial damage (Kerenyi et al., 1985). Our group also reported the strong colonization to human type-1 collagen immobilized on microtitre plate by indigenous *L. plantarum* strains, the binding capability of Lp91 with collagen (177.66 ± 11.50 CFU/well) and percentage hydrophobicity (39.49 ± 6.12%) was consistently better amongst other test cultures (Yadav et al. 2013). The hydrophobicity has been positively correlated with adhesion abilities and competitive inhibition

in several studies, suggesting a good relationship between in vitro adhesion and in vivo colonization (Collado et al. 2007; Deepika and Charalampopoulos 2010; Duary et al. 2011). In another study conducted in our laboratory, we investigated the quantitative binding of the selected *L. plantarum* strains on immobilized mucin and fibronectin by enumeration of CFU count after platting on MRS agar. All the test cultures were adhered to immobilized mucin and fibronectin at different levels. Selected strains have better binding with fibronectin as compared to mucin (unpublished data).

However, infectious diseases caused by *Lactobacilli* are very rare and the role of ECM binding in infective endocarditis (IE) remains tentative. Many pathogens adhere to ECM (Patti et al., 1994; Westerlund & Korhonen, 1993) However, adherence of LAB to subintestinal ECM can, on the other hand, be a probiotic characteristic. Such an adherence may protect the host against bacterial invasion at damaged epithelia where the ECM has become exposed.

Adhesion to Mucus

Mucus is a gel-like structure secreted by the goblet cells and mucosal glands, and covers the intestinal epithelium. The main structural components of mucus are large molecules (>200 kDa) called mucins that are polymers of a highly glycosylated protein monomer and held together by disulfide bonds (Zhu et al., 2010; Bell et al., 2001; Mantle et al., 1984). Other components of mucus include protein, lipid, DNA and membrane fragments from epithelial cells (Qin et al., 2008). The mucus layer diminishes the access of harmful bacteria to intestinal tissue surfaces by providing a physical barrier that pathogens must penetrate before invading the intestinal epithelium (Crater & Carrier, 2010; Mantle & Husar, 1994; Cover & Aber, 1989). Degradation of mucus by mechanical forces or enzymes releases partially degraded or denatured

mucins into the intestinal lumen which may further enhance mucosal protection by binding to bacterial adhesins (Jia et al., 2010; Mantle & Husar, 1994). As the outermost luminal layer mucus is the first intestinal component or surface that LAB are likely to contact before they reach epithelial cells. Hence, it can have a substantial role in the colonization of intestinal surfaces. There are a number of studies that report binding of LAB strains to mucus from animals and humans (Mackenzie et al., 2010; Kankainen et al., 2009). Attachment of probiotic bacteria to specific sites on intestinal mucosa cells might lead to competitive exclusion of pathogens and modulation of host cell responses. Proteins that are expressed on the bacterial cell surface are considered to play an important role in such interactions (Boekhorst et al., 2006). The reduced adhesiveness of *Lactobacilli* treated with proteinases has led to the hypothesis that proteinaceous molecules mediate the adhesion of *Lactobacilli* in the host intestine (Sun et al., 2007; Greene & Klaenhammer, 1994; Fuller, 1989). The involvement of carbohydrates and lipoteichoic acids in the adherence of *Lactobacilli* to intestinal and genital epithelia has also been reported (Neeser et al., 2000; Granato et al., *1999*; Adlerberth et al., *1996*) but the adhesive structures have not been identified. Overall, the various results suggest that *Lactobacilli* adhere to host tissues via mechanisms that vary in different species and to understand these underlying mechanisms, several model systems have been proposed.

Model Systems for Probiotic Adhesion

The interactive action between the microflora and intestinal mucosa requires that *in vitro* and *in vivo* methods to study this relationship (Van den Abbeele et al. 2012; Ouwehand et al. 2001; Bernet et al. 1994). Unfortunately, the ecosystem of the human GIT represents numerous obstacles for the study of associated microflora and the interaction between the microflora and

mucosa. Efforts to avoid these obstacles have led to the development and utilization of numerous *in vitro* cell culture models, *in vivo* animal systems, and *in silico* mathematical models. Although an accurate replica of the human gastrointestinal environment has not been developed for adhesion studies, but through the conscientious use of model systems, experiments can be performed that provide insight to specific intestinal relationships of interest.

In vitro model systems of the human GIT allow researchers control over specific conditions to examine a single parameter in detail. Most *in vitro* studies of the relationship between bacteria and intestinal mucosal surfaces use cultures of enterocytes or explanted sections of intestinal mucosa. These model systems allow the study of both the bacterial and host factors involved with bacterial association with the mucosal surface of interest

Caco-2 Cell Line

One of the more common enterocyte cell lines used for bacterial adherence studies is the Caco-2 model. Originally studied as a gastrointestinal tumor cell line for performing studies on cancer mechanisms, Caco-2 cells were found to be unique in their ability to spontaneously differentiate in culture (Grasset et at. 1984), although colonic in origin Caco-2 cells express several morphological and enzymatic features of small intestinal enterocytes upon differentiation. These cells grow with a cylindrical polarized morphology in a monolayer expressing brush border microvilli and small intestinal hydrolase activity on the apical surface with tight junctions between cells (Sambuy et al., 2005). For intestinal transport and bacterial invasion studies, these cells are grown on permeable filter supports that allow access of ions and nutrients to both sides of the monolayer. Bacterial adhesion studies generally use cells grown on multi-well plates specially treated for cell culture (Stetinova et al., 2010). In culture, the Caco-2

is a heterogeneous population of cells with different morphologies. Clonal cell lines have been created from the parental Caco-2 culture (ATCC HTB-37) to improve the homogeneity of the population or isolate a specific desired phenotype from a subpopulation. For example, the C2BBe cell line was sub-cultured for more homogeneous brush border expression comparable to *in vivo* human intestinal enterocytes (Leonard et al., 2010; Huang & Adams, 2003). Although Caco-2 cells are widely used for bacterial adhesion studies because of their morphological and functional similarity to human small intestinal enterocytes, the inherent heterogeneity of the Caco-2 line combined with different cultures conditions make the comparison of results between laboratories difficult. While Caco-2 cells are appropriate as an intestinal enterocyte model, they are not able to accurately replicate the mucus layer of the intestinal mucosal (Nissen et al., 2009).

HT29 Cell Line

Another colonic carcinoma cell line HT29 has been sub cultured into distinct populations with varied morphologies and mucin expression. Following exposure to either 5-fluorouracil (HT29- FU) or methotrexate (HT29-MTX), subpopulations were isolated that showed varied morphology and mucus secretions. The availability of mucin specific sub clones allows researchers to study the adhesion of bacteria to either colonic or intestinal mucin. The availability of immunoassays for specific mucin types provides a mechanism for studying mucin induction by intestinal bacteria. For example, certain adhesive *Lactobacillus* species have the ability to induce the expression of MUC3. The MUC3gene product is a secreted small intestinal mucin with the ability to inhibit enteric pathogen adherence (Jia et al., 2010; Hatakka et al., 2007). Mack et al. (2003) reported the induction of the intestinal mucin MUC2 and MUC3 from HT29 cells, but not from the non-intestinal HEp-2cells. MUC2 and MUC3 were highly induced

in HT29 cells following exposure to both *L. plantarum* 299v and *L. rhamnosus* GG, and both the mucins were able to inhibit the adherence of pathogenic *E. coli* species.

Animal Models

In vitro cell lines are commonly used due to their relatively low cost, their ability to be controlled for targeted experimentation and their ease of access and manipulation. However, the lack of realism of these cell lines makes animal models more illustrative of the human gastrointestinal environment. Although ethically restrictive, animal models offer a more accurate representation of the complexity present in the human GIT. For example animal models more closely replicate the integration of mucosal and luminal surfaces along with the immune response naturally established in the GIT of humans (Coconnier et al., 1992). Conventional animal models present the highest degree of realism and fewest ethical restrictions, but the complexity of factors in the gastrointestinal tract limit their use in bacterial adhesion studies. Germ free and gnotobiotic animals are not as representative of the human GIT as conventional animals but experimental conditions can be more easily controlled (Fritz et al 2013). The lack of a complex flora makes the interpretation of data obtained from bacteria-host interactions in gnotobiotic animals more straight forward. Use of germ-free animals is essential for determining the effect of a single bacterial population on normal development, establishment and maintenance of the mucosa-associated immune system and epithelial-cell functions. Experiments in germfree animals illustrate the importance of indigenous microflora in protecting against intestinal colonization by exogenous bacteria (Kim et al., 2010; Stetinova et al., 2010; Coconnier et al., 1992). More recently *in silico* analyses utilizing mathematical models of bacterial adhesion to epithelial cells and mucosal surfaces are being employed. In one such study Lee et al. (2000)

created a mathematical model of the adhesion of *L. rhamnosus* GG, *L. casei* Shirota, and *E. coli* TG1 to Caco-2 cells. The model was based on predetermined numbers of bacterial receptors on the Caco-2 cells and bacterial efficiency of binding to those receptors. Although the mathematical model was used to estimate the competitive exclusion properties of the *Lactobacilli* against *E. coli,* it was clear that "wet lab" experimentation was necessary for quantitation of the adhesive abilities of these strains. These and other mathematical models allow researchers to study the principles of known interactions within a specified model system.

The effects of specific probiotics or other compounds cannot be accurately estimated. However, with the integration of data from a growing body of knowledge on bacterial adhesion mechanisms, *in silico* analysis could move closer to representing *in vivo* relationships. Animal models offer insight into the accuracy of hypotheses in a controlled, yet complex system. *In vitro* cell culture models use a reductionist approach to study specific relationships in a manageable and reproducible environment. Integration of data from other models into mathematical models allows for the relatively rapid estimation of general relationships between bacteria and the intestinal environment (Laparra & Sanz 2009; Boureau et al., 2000). However, human models are the final, integrated step in assessing theoretical predictions. Unfortunately, ethical restrictions and lack of environmental control make human trials both expensive and difficult to perform. Nevertheless, use of these models have confirmed that adhesion to intestinal epithelium is facilitated by several important factors including some surface layer proteins such as mucus/mucin binding protein (Mub), collagen binding protein (Cbp), fibronectin binding protein (Fnbp) and Laminin etc. Characterization of these proteins thus becomes important to fully understand their role in affecting adhesion capability of a bacterial strain.

Surface Layer Proteins

Surface layer proteins represent 10-15% of the total protein of the bacterial cells. The genes encoding the surface layer proteins are diverse but their amino acid compositions are similar. Surface layers serves as protective coats, cell shape determinants, traps of other molecules and ions, virulence factor (for pathogenic species) and adhesion sites for exoenzymes and host cells (Jaaskelainen et al., 2008). The proteinaceous surface layers are composed of subunits of single protein or glycoprotein covering the entire cell as the outermost envelope (Fig. 2), with molecular masses ranging from 40 to 200 kDa (Chen et al., 2009). Surface layers are attractive candidates for use in nanotechnological applications since they form a regular paracrystalline array with high periodicity (Pum & Sleytr, 1999; Sleytr & Beveridge, 1999; Sleytr & Sara, 1997) that reflected their anchoring mechanism to Gram +ve cell surface.

The surface layer genes have been cloned and characterized from several *Lactobacilli* species viz. *L. acidophilus* (Boot et al., 1993), *L. gallinarum* (Hagen et al., 2005), *L. helveticus* (Gatti et al., 2005; Callegari et al., 1998) and from *L. brevis* (Jakava et al., 2002; Vidgren et al., 1992). Previously, *L. johnsonii* and *L. gasseri* were proposed to lack an S-layer (Boot et al., 1996) but Ventura et al., (2002) identified the protein called aggregation promoting factor from these species as an S-layer like protein having amino acid composition and physical properties similar to *Lactobacillus* S-layers. Despite their similar amino acid composition such as a low content of cysteine and methionine as well as a high content of hydrophobic amino acids and hydroxyl amino acids, the S-protein primary sequences are conserved only in closely related species. When the phylogenetic trees constructed on the basis of 16S rRNA or tuf gene sequences of a set of *L. acidophilus* related organisms, including strains of the novel *L.*

suntoryeus species were compared with those constructed on the basis of surface layer protein genes of the same species, the novel strains no longer grouped together indicating strong selective pressure forcing the diversification of S-layer protein genes within *L. acidophilus* related organisms as well (Cachat & Priest, 2005). In a similar analysis, however, the comparison of phylogenetic trees based on 22 deduced *Lactobacillus* surface layer protein sequences and 16S rRNA sequences of corresponding *Lactobacillus* species, available revealed a similar overall clustering of strains. S-layer proteins form ubiquitous cell envelope structures that are composed of numerous identical subunits held together through interactions with the underlying cell surface (Jaaskelainen et al., 2008). Surface layer proteins have complex hydrophobic and hydrophilic properties. Bacterial surface layer adhesive proteins have been explored as a potential mediator of *Lactobacillus* adhesion to intestinal epithelial cells (Johnson et al., 2007). Several studies have explored the role of S-layer protein in adhesion of *Lactobacillus* to intestinal epithelial cells (Golowczyc et al., 2007) and were found to have an important role in the underlying mechanism of bacterial adhesion thus benefiting the host. Liu et al., (2010) investigated the use of novel surface layer protein from probiotic bacteria for the treatment of inflammatory bowel disease and other gastrointestinal disorders and concluded that surface layer protein purified from the probiotic bacteria protected intestinal epithelial cells from enteropathogenic *E. coli* induced injury through a mechanism involving extracellular signal regulated kinase. It has now become apparent that not only these surface layer proteins play a major role in facilitating adhesion of bacterial cells with ECM but they can also be used as anti-adhesion agents for the prevention of infectious diseases which is indeed a potential probiotic trait (Yadav et al. 2013; Horie et al., 2002; Toba et al., 1995). Among several forms of surface layer proteins, collagen binding

protein is one of the important surface adhesin if *lactobacilli* interact with collagen that is an adhesion capability as a desirable characteristic for probiotic.

Collagens form a super family of proteins that are the most abundant ECM molecules. They are expressed in all tissues of the human body and are involved in many important functions that support the architecture, strength and development of tissues and proliferation, migration and differentiation of cells after attachment. Collagens play an important role in tissue preparation, acting as a network that helps in the sealing of wounds. All collagen molecules form supramolecular aggregates that are stabilized in part by triple helical domain interactions (Prockop et al., 1998; Fessler & Fessler, 1978). Various human pathogenic bacteria exhibit specific adhesiveness to the mammalian extracellular matrix proteins i.e. collagens. Adherence to collagens is generally thought to promote bacterial colonization at damaged tissue sites, such as wounds, and is essential in enterobacterial invasion from the intestine into the circulation in orally infected mice. The majority of *Lactobacillus* isolates of human or animal origin express adhesiveness to collagens (Sillanpaa et al., 2000) which is considered as an important probiotic property.

Collagen Binding Protein

Several investigators found that majority of the *Lactobacillus* isolates have the ability to bind to collagen. It seems that many *Lactobacilli* express multiple adhesin types interacting with these abundant tissue proteins. Collagen binding proteins have been identified and characterized in some bacterial species (Velez et al., *2007*). Size and binding ability of collagen binding proteins varied in different *Lactobacillus* species. Binding of immobilized collagen-I (Cn-I) and fibronectin (Fn) by *L.* acidophilus CRL 639 depended on cell surface proteins. Collagen -1 binds

to 45 and 58 kDa proteins where as fibronectin (Fn) binds to a 15-kDa protein of *L. acidophilus* (Lorca et al., 2002). *L. crispatus* JCM 5810 adhered to collagen-rich regions in the colon tissue of chicken, the natural host for JCM 5810 that supports the notion that collagen binding represents a true tissue-binding property of *L. crispatus* (Sillanpaa et al., 2000). Collagen binding protein (CbsA) gene of *L. crispatus* strain encoding a protein that mediates adhesiveness to collagens was characterized and expressed in *E. coli*. The *cbsA* open reading frame encoded a signal sequence of 30 amino acids and a mature polypeptide of 410 amino acids with typical features of a bacterial S-layer protein. The *cbsA* gene product was expressed as a *His* tag fusion protein purified by affinity chromatography and shown to bind solubilized as well as immobilized type I and IV collagens. Other *Lactobacillus* S-layer proteins, *SlpA*, *CbsB* and *SlpB* bound collagens only weakly and sequence comparisons of *CbsA* with these S-layer proteins were used to select sites in *cbsA* where deletions and mutations were introduced. Analysis of these molecules revealed the major collagen-binding region within the N-terminal 287 residues and a weaker type I collagen-binding region in the C terminus of the *CbsA* molecule. Strain JCM 5810 was found to contain another S-layer gene termed *cbsB* that was 44% identical in sequence to *cbsA*. RNA analysis showed that *cbsA* but not *cbsB* was transcribed under laboratory conditions (Sillanpaa et al., 2000). Another collagen-binding protein (*cnb*) of 31 kDa was isolated from *L. reuteri* NCIB 11951 along with cbsA. The two proteins cross-reacted immunologically indicating that they are related proteins but the N-terminal amino acid sequences show low homology (Aleljung, et al., 1994).

The *cnb* gene has been expressed in *E. coli* and the resulting recombinant protein was shown to bind solubilized type I collagen (Roos et al., 1996). Twenty N-terminal amino acids of

a 29 kDa surface protein isolated from *L. fermentum* RC-14 shared 100% identity with the N-terminus of Cnb, but the similarity for the rest of their sequences is not yet known. The protein was suggested to inhibit the adhesion of *Enterococcus faecalis* to polystyrene and to bind collagen (Howard et al., 2000) and thus could be a related adhesin molecule of *L. fermentum*.

A large (358 kDa) surface protein, *Mub*, from a pig intestinal isolate of *L. reuteri* 1063 mediated bacterial binding to pig and hen intestinal mucus. The deduced amino acid sequence of the *Mub* gene contained two types of large amino acid repeats (Roos et al., 2000). One with a chitin-binding domain (Yuen et al., 1990) another with a fibronectin-binding domain (Christie et al., 2002) and seven proteins with domains predicted to be involved in adherence to mucus. These putative mucus-binding proteins contained either copies of the mucus binding protein (Mucbp) domain or copies of the larger mucin binding (Mub) domain. While, the Mucbp domain was not only found in lactic acid bacteria but also in *Listeria* species, the Mub domain appears to be unique for lactic acid bacteria (Boekhorst et al., 2006). The collagen binding domain is found in a wide range of bacteria; while, the fibronectin binding and chitin-binding domains are present in proteins from both eukaryotes and prokaryotes. Collagen binding proteins are required for adhesion of *Lactobacilli* to human GI track surface. However, only three bacterial collagen binding domain structures are known the *Staphylococcus aureus* collagen-binding domain, (Symersky et al., 1997), the CBD of *Clostridium histolyticum* class I collagenase (Wilson et al., 2003) and *Yersinia enterocolitica YadA* domain (Kristin et al., 2006).

In another study, we have reported that purified 72 kDa cbp from *L. plantarum* 91 strain demonstrated strong anti-adhesion potential against gut pathogen *E. coli 0157:H7* in in-vitro study. The purified Cbp protein inhibits 59.71% adhesion of *E. coli 0157:H7* strain on

immobilized collagen (Yadav et al. 2013). The lack of knowledge about molecular adhesion machanisms of *Lactobacilli* with the help of these surface layer proteins does not allow us to draw firm conclusions about significance of these observations. However, new data obtained at genetic level as regard to protein secreted by *Lactobacilli* and their ability to adehere to mucus (Yadav et al., 2013; Duary et al. 2012b) may provide a breakthrough.

Conclusion and future perspectives

Several surface layer proteins have been identified and characterized in *Lactobacillus* species. Surface layer protein from *Lactobacillus* species act as adhesins that mediate colonization to gastrointestinal epithelial cells. It is evident that surface layer protein of *Lactobacilli* have important role in facilitating bacterial tissue adherence and cell surface hydrophobicity is a direct reference of adhesion ability of a *Lactobacilli* strain with gut enthrocytes. Additionally, different studies from our lab and others reviewed here suggested that multiplex PCR assays and also *cbp* based biomarkers can be developed for rapid screening of cbp positive probiotic *Lactobacilli* strains.

Considering the intricacy of host-microbe interactions involving host-cell signaling, immune-modulation and regulation pathways, it is highly unlikely that single-effector molecules regulate the entire host response. These molecules probably have extensive responsibilities and functions in addition to playing crucial roles as building blocks of the bacterial cell wall. Knowledge of the molecular mechanisms underlying the physiological characteristics of *Lactobacilli*, and identification as well as validation of responsible molecules complemented with simultaneous studies for their corresponding receptors in the host cells, will strengthen the

concept of strain specificity and contribute to the development of strains with enhanced health benefits for humans.

Also as food-grade probiotic organisms, *Lactobacilli* are excellent candidates for applications like live oral vaccines, where their ability to survive in the gastrointestinal tract could be utilized and the surface layer proteins could be used as carriers of antigens or other medically important molecules. The polymeric nature and inherent adjuvant properties of surface layers can be utilized in this approach. Further, immobilization of recombinant surface layer proteins conjoined with the display of foreign molecules in the surface layer forms the basis for the development of different solid-phase reagents, such as diagnostic tools, biocatalysts and biosensors. The increasing knowledge about the functionality of *Lactobacillus* surface layer proteins, as well as the developing tools to genetically manipulate these organisms, will also contribute in improving global human health programs.

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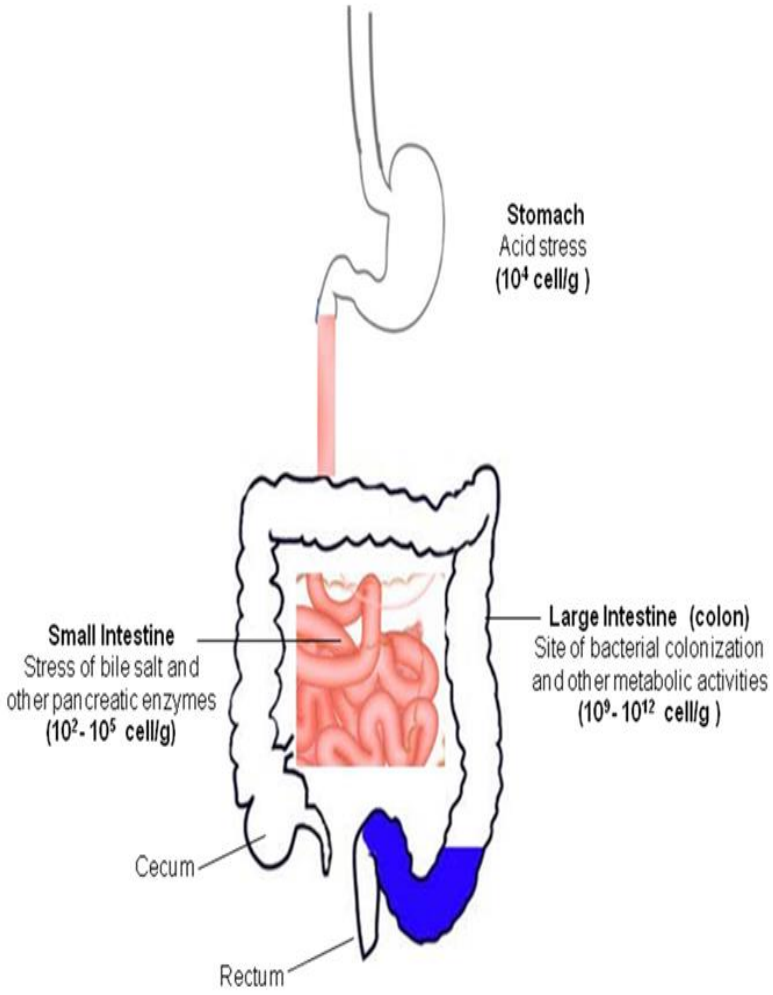

Fig.1. Schematic representation of the human intestine indicating its different regions and the overall sizes of the residing bacterial populations

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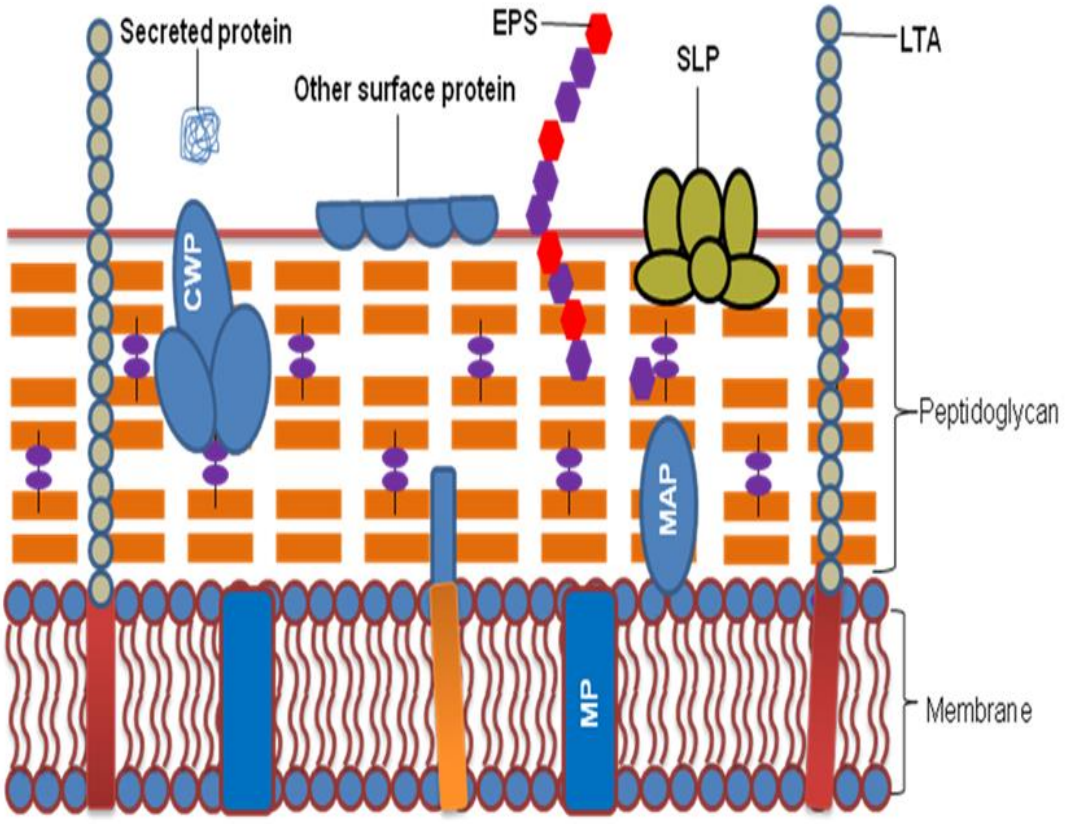

Fig 2. Schematic representation of the Gram-positive cell wall. Specific components of the cell Wall are indicated, including the peptidoglycan layer, wall- and lipoteichoic acids (WTA and LTA), exopolysaccharides (EPS), and proteins that can be presented to the cell surface in a variety of ways, including secretion to the environment (SP), membrane proteins (MP), cell-wall-associated proteins (CWP), membrane-anchored proteins (MAP), and surface layer proteins (SLP).

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