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Adherence of lactobacilli to intestinal mucosa and their antagonistic activity against pathogens

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Introduction

The study and therapeutic use of various beneficial bacteria for health purposes for both humans and animals is termed Probiotics, literally "for life", the definition highlighting the significant role of these friendly bacteria in influencing our health within the complex metabolic, biochemical and nutritional cycles of the human body [43, 76]. Probiotics may have antimicrobial, immunomodulatory, anticarcinogenic, antidiarrheal, antiallergenic and antioxidant activities [33, 37, 39].

Among these, there are to be mentioned: the competition against harmful micro-organisms including *Candida*, preventing the colonization of pathogens through the production of inhibitory substances including acids, hydrogen peroxide and natural antibiotics; the enhancement of digestion of lactose (milk sugar); reduction in blood cholesterol levels; the enhancement of the immune system, including enhanced macrophage activity; decrease in the risk of colon cancer by detoxification of carcinogenic compounds and toxic substance [85]; the deactivation of direct anti-tumour activity of certain strains; the reduction in liver toxicity; enhancement of peristalsis, digestion, regularity and re-absorption of nutrients; the promotion of a healthy digestive tract colonization in infants; the enhancement and balance of estrogen levels, prevention of osteoporosis through increased calcium uptake; protection against food poisoning, travellers' diarrhoea, allergies, skin problems; the enhancement of vitamin status (B, K), digestion of proteins, fats, carbohydrates.

The two main groups of micro-organisms which have been shown to be therapeutically beneficial as probiotics are lactobacilli, represented by the two most important probiotic *Lactobacillus* strains, *L. acidophilus* and *L. bulgaricus* [3].

L. acidophilus inhabits the human small and large intestine, and it is also found in the mouth and vagina. It suppresses hostile invaders including *Candida albicans* through the production of natural antibiotics and other inhibitory substances such as lactic acid, and H_2O_2 [4].

L. bulgaricus, found in yoghurt and cheese, can encourage a more acidic environment by producing H_2O_2 and antibiotic substances to inhibit harmful bacteria.

Other lactobacilli species and strains used as probiotics include *Lactobacillus brevis, Lactobacillus casei, Lactobacillus cellobiosus, Lactobacillus crispatus, Lactobacillus curvatus, Lactobacillus fermentum, Lactobacillus* GG *(Lactobacillus rhamnosus* or *Lactobacillus casei* subspecies *rhamnosus)* (69), *Lactobacillus gasseri, Lactobacillus johnsonii, Lactobacillus plantarum* and *Lactobacillus salivarus* (77).

The *Lactobacillus plantarum* 299v strain originates in sour dough. *Lactobacillus plantarum* itself is of human origin. Other probiotic strains of *Lactobacillus* are *Lactobacillus*

acidophilus BG2FO4, *Lactobacillus acidophilus* INT-9, *Lactobacillus plantarum* ST31, *Lactobacillus reuteri, Lactobacillus johnsonii* LA1, *Lactobacillus acidophilus* NCFB 1748, *Lactobacillus casei Shirota, Lactobacillus acidophilus* NCFM, *Lactobacillus acidophilus* DDS-1, *Lactobacillus delbrueckii* subspecies *delbrueckii*, *Lactobacillus delbrueckii* subspecies *bulgaricus* type 2038, *Lactobacillus acidophilus* SBT-2062, *Lactobacillus brevis, Lactobacillus salivarius* UCC 118 and *Lactobacillus paracasei* subspecies *paracasei* F19.

Lactobacillus plantarum 299v, which is derived from sour dough and which is used to ferment sauerkraut and salami, has been demonstrated to improve the recovery of patients with enteric bacterial infections. This bacterium adheres to reinforce the barrier function of the intestinal mucosa, thus preventing the attachment of pathogenic bacteria to the intestinal wall, while *Lactobacillus* GG was found to eradicate *Clostridium difficile* in patients with relapsing colitis [27].

Lactobacillus GG has also been shown to inhibit chemically induced intestinal tumors in rats by binding to some chemical carcinogens, and scavenging superoxide anion radicals, inhibiting lipid peroxidation and chelating iron *in vitro* (***). The iron chelating activity of *Lactobacillus* GG may account, in part, for its antioxidant activity. Other lactic acid bacteria, including strains of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* have also shown antioxidative ability [70]. Mechanisms include chelation of metal ions (iron, copper), scavenging of reactive oxygen species and reducing activity.

Lactobacillus casei has been demonstrated to increase levels of circulating immunoglobulin A (IgA) in infants infected with rotavirus. This has been found to be correlated with a shortened duration of rotavirus-induced diarrhea. *Lactobacillus acidophilus* and *Bifidobacterium bifidum* appear to enhance the nonspecific immune phagocytic activity of circulating blood granulocytes [14, 18, 22]. This effect may account, in part, for the stimulation of IgA responses in infants infected with rotavirus. In healthy individuals, *Lactobacillus salivarius* UCC118 and *Lactobacillus johnsonii* LA1 were demonstrated to produce an increase in the phagocytic activity of peripheral blood monocytes and granulocytes. Also, *Lactobacillus johnsonii* LA1, but not *Lactobacillus salivarius* UCC118, was found to increase the frequency of interferon-gamma-producing peripheral blood monocytes.

Adherence and colonization of intestinal mucosa by lactobacilli

The antimicrobial activity of probiotics is thought to be largely accounted for by their ability to colonize the colon and reinforce the barrier function of the intestinal mucosa [11]. Probiotics, such as *Lactobacillus bulgaricus,* which do not adhere as well to the intestinal mucosa, are much less effective against enteric pathogens [47].

Adhesiveness is probably one of the main factors to consider when assessing a strain's probiotic potential [15, 16, 24, 42, 50, 53]. In the gastrointestinal tract, adhesiveness means adhesion to cells, mucosa, and other bacteria, because for an inserted probiotic to become colonized in an environment that has more than 400 microbial species, adhesion to bare surfaces is unusual [51, 71]. Adherence of these organisms to mucosal structures is generally believed to facilitate colonization and persistence of lactobacilli and other bacteria in the normal intestinal population [5, 46, 48]. There are numerous ways to determine the adhesiveness of bacteria, which is one of the properties required of probiotic products that provides a scientific basis for the screening and selection of probiotics that compete with selective groups of pathogens for adhesion to intestinal surfaces [42, 58, 68].

Little is known about the surface properties of *Lactobacillus* that mediate attachment to human epithelial cell surfaces. Different studies have looked at the specific features of the surface of *Lactobacillus* strains [35]. These features include the effect of *Lactobacillus* on the

hemagglutination of rabbit, sheep, bovine, guinea pig and human erythrocytes, its surface hydrophobicity, slime production and the adherence of *Lactobacillus* to two different epithelial cell lines. These properties were compared by using electron microscopy on the cell surface. In a few *Lactobacillus* strains, surface properties such as hemagglutination of human OP_1 erythrocytes, the presence of extracellular slime material and their high degree of hydrophobicity appeared to be related to one another. The most surface-active strains of *Lactobacillus* adhered to both enterocytes and to vaginal cells well. These features seem to be closely related; however, different adhesions and various mechanisms of attachment may play a role in the adherence of different *Lactobacillus* strains to epithelial surfaces.

There are studies describing the ability of the lactobacilli to adhere to enterocytic epithelial cells *in vitro*, founding that the bacteria adhered at higher levels to differentiated rather than undifferentiated epithelial monolayers [7]. Given these results, Caco-2 cell lines have been widely used to measure the adhesiveness of bacteria in relation to the intestinal tract [34]. When these cell lines and flow cytometry were combined, adhesion of lactobacilli was found to vary; isolates from some commercial products (such as LC1 [Nestle, Lausanne, Switzerland] and GG [Valio, Helsinki, Finland]) were better than those from others (Lactophilus)] [38]. In a study of adhesion of lactobacilli to mucus from newborns and adults, the strain *Lactobacillus rhamnosus* GG adhered best. Perhaps more important, adhesion of the strains to adult mucus was high. The authors suggest that not all strains may be ideal for newborns and adults.

Other studies showed that stationary phase lactobacilli were found to adhere to eukaryotic HT-29 and Caco-2 epithelial cells at greater levels than log phase bacterial cells.

The molecular mechanisms by which lactobacilli adhere to epithelial cells remain currently unknown [79].

Several studies have suggested that *Lactobacillus* adherence is mediated by proteins, while others have suggested a role for lipoteichoic acid and carbohydrate [17, 20, 26].

The potential involvement of surface/exposed protein(s) as bacterial adhesion(s) in lactobacilli was proven by studies showing that pretreatment of the *Lactobacillus* cells with proteolytic enzymes cancelled attachment. SDS-PAGE (denaturing) techniques made the proteolytic treatment result in the degradation of a cell wall-associated protein of approximately 84 kDa [31]. The proteinaceous factor was purified by both anion-exchange chromatography and by gel extraction after SDS-PAGE electrophoresis, and under *in vitro* assay conditions proved capable of adherence and significant inhibition of bacterial attachment to enterocytic epithelial cells.

The protease treatment of the bacterial cells has either not affected or enhanced the adherence of *L. gasseri* ADH. Periodate oxidation of bacterial cell surface carbohydrates significantly reduced adherence of *L. gasseri* ADH, moderately reduced adherence of L. acidophilus BG2FO4, and had no effect on adherence of *L. acidophilus* NCFM/N2. These results indicate that *Lactobacillus* species adhere to human intestinal cells via mechanisms which involve different combinations of carbohydrate and protein factors on the bacterial cell surface.

Studies on *Lactobacillus acidophilus* aggregation and adhesiveness have been demonstrated that *L. acidophilus* has the ability to establish in the human gastrointestinal tract, by exhibiting a strong self-aggregating phenotype and manifests a high degree of hydrophobicity determined by microbial adhesion to xylene, properties mediated by proteinaceous components on the cell surface [36].

Our research showed that all LAB strains exhibited a strong ability to attach to HeLa cells, showing aggregative and localized adherence pattern. The adherence rate was tested comparatively for bacterial mid-logarithmic phase cultures as well as for washed bacterial cells re-suspended in Eagle MEM. Paradoxically, the intensity of the adherence rate was

higher when live bacterial cultures were used, as compared to bacterial washed sediments, meaning that the intensity of adherence to the cellular substrate is depending on the gradient of some bacterial compounds secreted and accumulated in the culture medium directly or indirectly involved in the cell to cell signaling (Lazar *et al.*, 2004).

Cell surface hydrophobicity plays a role as the organisms approach a surface (for example that of a cell, mucus, or biomaterial). Previous studies have shown that lactobacilli can have a range of hydrophobic surface properties and that both hydrophobic and hydrophilic organisms can adhere to epithelial cells, probably *via* different interlocking mechanisms. The finding that a particular strain of *Lactobacillus fermentum* subspecies *cellobiosus* had a highly hydrophobic surface similar to that of *Salmonella gallinarum* implied that the *Lactobacillus* strain could block colonization of *S. gallinarum* [26].

The bacterial adhesion to hydrocarbons (BATH) test was adopted to screen lactic acid bacteria for cell surface hydrophobicity. The test uses the different distribution of hydrophobic and hydrophilic strains in a solvent (n-hexadecane)/water system which is recorded by optical density determination [64].

Heat and protease treatment of bacteria of high surface hydrophobicity, including self-aggregating strains in phosphate-buffered saline, showed a drastic decline in this surface property. Cultures of selected strains grown in liquid media rich in carbohydrates did not affect their hydrophobic cell surface character. Therefore, it seems less likely that carbohydrate capsule polymers are the major determinants of intestinal colonization of lactobacilli.

The degree of hydrophobicity predicted the adhesion of *L. rhamnosus* GG to Caco-2 cells. *L. rhamnosus* GG, however, was able to compete with *Escherichia coli* and *Salmonella* spp. of low hydrophobicity and high adhesion–receptor interaction for adhesion to Caco-2 cells. The interference of adhesion of these gastrointestinal bacteria by *L. rhamnosus* GG was probably through steric hindrance and the degree of inhibition was related to the distribution of the adhesion receptors and hydrophobins on the Caco-2 surface [75]. A Carbohydrate Index for Adhesion (CIA) was used to depict the binding property of adhesions on bacteria surfaces. CIA was defined as the sum of the fraction of adhesion in the presence of carbohydrates, with reference to the adhesion measured in the absence of any carbohydrate. The degree of competition for receptor sites between *Lactobacillus casei* Shirota and GI bacteria is a function of their CIA distance. There were at least two types of adhesions on the surface of *L. casei* Shirota.

Binding of lactobacilli to extracellular matrix (ECM) proteins (collagen (Cn) human type I and IV, fibrinogen (Fb) and fibronectin (Fn) may be also involved in the colonization of the intestinal mucosa by lactobacilli [83].

Although the gastrointestinal tract is the main target of available probiotic cultures — and indeed most definitions of probiotics refer to intestinal site of action —probiotic organisms have applications at other sites, such as the urogenital tract, nasopharynx, wounds, and elsewhere. *Lactobacillus rhamnosus* GR-1, *Lactobacillus fermentum* RC-14, and *Lactobacillus fermentum* B-54 have antipathogen properties and colonize the intestine and vagina, conferring health benefits to women [62]. These strains have been found to adhere to vaginal cells, hemagglutinate red blood cells and produce biosurfactants.

Lactobacilli form the large part of vagina microbiota that generating a microenvironment usually capable of protecting the host from infectious diseases, including some that are sexually transmitted or which increase the risk of preterm labor [62, 74]. Nevertheless, statistically every woman will suffer from yeast, bacterial vaginosis, or urinary tract infection at some point in her life [72, 73]. A number of factors are believed to be important for lactobacilli to colonize the urogenital and intestinal tracts and reduce the risk of infection [8, 9]. Adhesion to cells and mucus is believed to be one of the main factors when it

coincides with survival of the organism and inhibition of growth and adhesion of pathogens [63].

Little is known about the mechanisms by which lactobacilli from the vaginas of healthy young women adhere to vaginal epithelial cells, although the variety of surface structures in these bacteria implies that a spectrum of adherence mechanisms may exist (2). Furthermore, self-aggregation may substantially increase the colonization potential of lactobacilli in environments with short residence times.

The study of adhesion with respect to the intestine has been done primarily with CaCo-2 cells, and the clinical relevance of the adhesion values has been questioned. It would require intestinal biopsies to fully correlate adhesion *in vitro* with those obtained *in vivo*. Even then, given the fact that dense biofilms of organisms coat the mucus and cells of the intestine, the level of adhesion to cells may not be as critical as an ability to adhere to and penetrate existing biofilms. For the vagina, it is easier to obtain cells and determine if *in vitro* data correlates with *in vivo* findings. Adhesions can be detected by hemagglutination reactions (essentially binding of bacteria to blood cells), and a classification system has been reported using hemagglutination to separate strains that would be potentially good probiotic agents from those that would not.

Both self-aggregation and adhesion may favor the colonization of the vaginal epithelium through the formation of a bacterial film that may contribute to the exclusion of pathogens from the vaginal mucosa.

Multiple components of the bacterial cell surface seem to participate in the adherence of the strains to vaginal epithelial cells. In *L. acidophilus* and *L. gasseri*, adherence involved proteins and carbohydrate (possibly a glycoprotein), while *L. jensenii* adherence seemed to depend exclusively on carbohydrates (6). In *L. gasseri* and *L. jensenii*, divalent cations, probably Ca^{2+}, were also involved in adherence, as judged by sensitivity to EDTA and EGTA. This diversity of adherence requirements was reported before, although it was for the digestive epithelium. Thus, *L. fermentum* adherence to mouse squamous epithelium was sensitive to chelating agents, while colonization of chicken tissue by *L. acidophilus* was not. However, the adherence factors seem to be different from those that mediate the self-aggregation of the strains; for example, *L. jensenii* self-aggregation depends on lipoproteins, while adherence to vaginal cells relies on carbohydrates.

Competition with pathogens in mucosal colonization

Knowledge of the predominant genera and species of the gastrointestinal microflora as well as determination of their levels and biochemical activities are essential in the understanding of the microbial ecology of the gastrointestinal tract. The normal resident gastrointestinal microflora contains many diverse populations of bacteria which play an essential role in the development and well being of the host [67]. By continual release of antibiotic proteins, specialized cells of the intestinal epithelium may influence the extracellular environment and contribute to mucosal barrier function. In addition to the host cell non-immune system of defense, bacteria of the resident gut microflora exert a barrier effect against pathogens. It was recently reported that *Escherichia coli*, one of the first bacterial genera that colonize the intestine of humans, display antimicrobial activity against salmonella infection [30, 32]. Other species of the endogenous human microflora such as bifidobacteria, a major species of the colonic microflora, and lactobacilli, a minor species of the gut microflora, exert antimicrobial activity by producing secreted antimicrobial substances [23, 41, 45].

During the past decades, the beneficial effect of specific lactic acid bacteria (LAB) strains in preventing or treating intestinal disorders has been substantiated by well-controlled clinical trials [21]. Increasing evidence, including human studies, is also supporting the

immunomodulatory role attributed to given lactic acid bacterial strains [66]. To gain further insight into the mechanism by which resident bacteria of the human microflora could exert a protective role against enterovirulent pathogen induced cellular damage, it was examined the activity of a *Lactobacillus acidophilus* strain isolated from the resident adult human microflora for which the production of secreted antimicrobial substances has been well established both *in vitro* and *in vivo* [65].

To infect host cells, microbial pathogens use very sophisticated mechanisms of pathogenicity. Many enteroadherent and enteroinvasive pathogens hijack the host cell signal transducing pathways and/or target the host cytoskeleton molecules that play a pivotal role in cell architecture, thus promoting development of diarrhoea. It is well known that Ca^{2+} regulated events play a key role in the disassembly brush border organization leading to functional disorders promoting diarrhoea. It was established that the mechanism by which the strain LB by its SCS (Spent Culture Supernatant) exerts a protective effect against the one Enteropathogenic *E. coli* (EPEC) strain is an antagonistic activity results from interference with EPEC induced cell signaling. An identical mechanism of action — that is, inhibition of the cross talk between a pathogen and target host cells — has previously been reported with antibiotics.

Few studies of the exclusion or reduction of pathogen adhesion have been reported recently. Lactobacilli were shown to produce anti-pathogen adhesion substances, the crude mixture of which is termed *biosurfactants*, and a recent study showed that this inhibitory effect can extend to a wide range of virulent pathogens [59]. Presumably, these primarily proteinaceous biosurfactants are produced in situ, perhaps aided by lower pH. Human phospholipids (referred to as biosurfactants, although this is incorrect because other bacteria can produce lipid biosurfactants and the term should be retained for bacterial, not human compounds) play a role in cell membrane fluidity and permeability, helping to prevent pathogen translocation. These lipids are dependent on fatty acids. It has been shown that a strain of *Lactobacillus plantarum* can increase fatty acid content in the intestine, adhere and interfere with pathogen adhesion and translocation (perhaps by competitive binding to mannose receptors), and eliminate nitrate.

It has been suggested that by combining ingestion of this strain with various fibers and polar lipids, the gastrointestinal mucosa can be reconditioned and the risk for infection can be reduced.

Because LAB are known to exist in the intestines and exert antibacterial activity on intestinal pathogens and because the stomach is a very harsh environment, with low pH and the presence of pepsin, most researchers have assumed that LAB cannot inhibit gastric pathogens.

Among the 100 LAB isolated in infant feces, several isolates that showed good inhibitory activity on the adherence of *Helicobacter pylori* to glycolipid spotted on a thin-layer chromatography plate were selected. One isolate, which showed the best inhibitory activity on the growth of *H. pylori* in a well test, was selected and characterized [25, 49]. When *H. pylori* was treated with the SCS (*Spent Culture Supernatant*), the cells changed from helical shape to coccoid shape and became necrotic, even after treatment with the neutralized SCS [10]. A similar coccoid shape was observed with *H. pylori* treated with amoxicillin; others have previously observed the same result after treatment with the SCS of *L. acidophilus*, and this form is known to result in a loss of infectivity. Because the neutralized SCS had the same effect, there must be a factor(s) for necrosis in addition to lactic acid and low pH.

The binding activity of nonviable LAB suggests that LAB binds directly to the stomach *via* a mechanism similar to that involved in the binding of LAB to the intestinal epithelial cells and that nonviable as well as viable LAB can prevent the adherence of *H.*

pylori to the stomach. Such LAB strains with dual inhibitory activity on *H. pylori*—bactericidal activity and prevention of adherence to gastric mucous cells—has the potential to be developed as a new probiotic for the stomach.

Lactobacilli are believed to interfere with genitourinary pathogens by different mechanisms [55, 80, 81]. The first is competitive exclusion of genitourinary pathogens from receptors present on the surface of the genitourinary epithelium [12, 44]. Second, lactobacilli co-aggregate with some uropathogenic bacteria, a process that, when linked to the production of antimicrobial compounds, such as lactic acid, hydrogen peroxide, bacteriocin-like substances, and possibly biosurfactants, would result in inhibition of the growth of the pathogen [52, 56, 57, 58, 82].

A lot of studies have demonstrated that the vaginal lactobacilli interfered with the adherence of genitourinary pathogens [28]. In this respect, it is interesting to note first that *Candida (C) albicans* and *Gardnerella vaginalis* adhered to vaginal epithelial cells, while *E. coli* and *S. agalactiae* did not [40]. Since the first two organisms produce pathology primarily at the vaginal level, while the others are just opportunistic pathogens, it may be deduced that adherence is an important virulence factor.

It is possible that *L. acidophilus* and *G. vaginalis* bind to the same receptors on the surfaces of vaginal epithelial cells [29, 85]. It appears that the affinity of *L. acidophilus* for those receptors is higher than that of *G. vaginalis*, as deduced by the displacement by *L. acidophilus* of adherent cells of *G. vaginalis.*

Finally, there are studies showing that the vaginal lactobacilli co-aggregated with all of the pathogens, with the exception of *S. agalactiae*, suggesting that this process is somewhat specific [60, 61]. The co-aggregation may well impede the access of pathogens to tissue receptors and in fact may be an alternative explanation for the lack of adherence of *C. albicans* and *G. vaginalis* to vaginal epithelial cells in the presence of lactobacilli [54, 78].

Our results showed that the original LAB cultures, washed bacterial cells, or culture supernatant largely reduced the attachment of the tested pathogenic strains to HeLa cells (5 to a 10% adherence rate of pathogenic bacteria in the presence of the tested LAB preparations), but compared to the original LAB cultures (under 1% adherence rate of pathogenic bacteria in the presence of LAB whole cultures), washed cells or culture supernatant inhibited the adhesion of tested pathogens to a lesser degree (Smarandache *et al.*, 2004; Lazar *et al*., 2004).

However, this inhibiting activity was recovered after the washed cells and the supernatant were recombined, with the recombined mixture having a similar inhibitory ability to that of the original LAB cultures (pathogenic bacteria not adhered to HeLa cells). This result may imply that the substances from both LAB cells and the spent culture supernatant contribute to the inhibition of adhesion of pathogens to the HeLa strains (Lazar *et al*., 2004).

The inhibitory effects of LAB on adherence capacity of pathogens have been attributed to the steric hindrance of binding sites, pH values, or certain components of the lysed cell. The results of our studies support the idea that LAB cells or the substances released in its culture might occupy the binding sites, although these binding sites are not necessarily targeting the same epitopes in pathogenic strains.

The culture pH has been suggested to be an important factor in the inhibition of adhesion of pathogens to the mucosa. In the current study, the inhibiting effect was not solely a pH effect, since considerable inhibitory action was demonstrated after washing the bacterial cells and re-suspending them in EagleMEM and adjusting the bacterial suspension used for inoculation of cell monolayer to pH 7.0 (Lazar *et al*., 2004).

In the case of culture supernatants, we have to mention that a strong lytic effect, that was not recovered when washed bacterial cells or original cultures were used, was noticed on pathogenic bacterial cells. This could be due to the presence of lytic enzymes in bacterial culture supernatants. Paradoxically, this lytic effect was not observed when original bacterial

cultures were used. These results are pleading for the existence of complex regulatory and signaling mechanisms present in original cultures, that are lost when bacterial supernatants are used. However, further in-depth studies on a large number of pathogenic strains are needed in order to confirm these results.

We have also demonstrated, by *in vivo* studies that, beside the local, antipathogenic effect manifested at intestinal level, the probiotic *Lctobacillus casei* GG strain exhibit also an immunoadjuvant effect, when administered against enteropathogenic bacteria, together with fimbrial antigens by oral route (Dima *et al.*, 2002). The immunostimulatory effect was not only local (at the Peyer's patches and intestinal lymph nodes level, but also systemic, highlighted by the lymphocyte proliferation in spleen) (Dima *et al*., 2000).

This brief review presented the existent data on the role of lactobacilli adherence in the colonization of different mucosal surfaces or their persistence in host ecosystems after administration as probiotics. However, *in vitro* tests showed that adherence is highly dependent on certain physiological conditions (e.g. pH conditions not found in natural host ecosystems), and no correlation was observed between the *in vivo* findings and adherence of a certain bacterial strain to epithelial cells or cell lines [3, 19]. So we have to take into account the prediction limits of the *in vitro* cell culture models which cannot predict the success of a strain as a colonizer of human mucosal surfaces. The knowledge of the natural colonization pattern of lactobacilli in human is still rudimentary and more research is needed to confirm the role of adherence in colonization and persistence.

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