CHAPTER II

LITERATURE REVIEWS

1. General Characteristics of *Lactobacillus*

1.1 Classification of *Lactobacillus*

The *Lactobacillus* is classified as genus *Lactobacillus* as given below [1].

Kingdom	Bacteria
Division	Firmicutes
Class	Bacilli
Order	Lactobacillales
Family	Lactobacillaceae
Genus	*Lactobacillus*

1.2 Biology of *Lactobacillus*

Lactobacilli are gram positive, catalase negative, non-spore forming rods or coccobacilli varying from long and slender, sometimes bent rods to short rods, often coryneform, chain formation is common. Some strains exhibited bipolar bodies, internal granulation or a barred appearance with the Gram reaction or methylene blue stain [1]. They are members of the lactic acid bacteria, a broadly defined group characterized by the formation of lactic acid as a sole or main end product of carbohydrate metabolism [50].

Colonies of lactobacilli on agar media are usually small (2-5 mm), with entire margins, convex, smooth, glistening and opaque without pigment. In rare cases,

they are yellowish or reddish. Some species form rough colonies [1]. Lactobacilli do not develop characteristic odors when grown in common media. However, they contribute to the flavor of fermented food by producing various volatile compounds, such as diacetyl and its derivatives and even H_2S and amines in cheese [1].

Lactobacilli are extremely fastidious organisms which adapted to complex organic substrates. They require not only carbohydrates as energy and carbon source, but also nucleotides, amino acid and vitamins. The various requirements for essential nutrients are normally met when the media contain fermentable carbohydrate, peptone, meat and yeast extract. Supplementations with tomato juice, manganese, acetate and oleic acid esters, especially Tween 80, are stimulatory or even essential for the most species. Therefore, these compounds are included in the widely used MRS medium [51]. With glucose as a carbon source, lactobacilli may be either homofermentative, producing more than 80% lactic acid as main product or heterofermentative, producing mixed products of lactic acid, carbon dioxide, ethanol and/ or acetic acid in equimolar amounts [50, 52]. The lactic acid formed by the various fermentation pathways possesses either L-or the D-configuration depending on the stereospecificity of the lactate dehydrogenase present in the cells [1].

Lactobacilli grow best in slightly acidic media with and initial pH of 4.5-6.4. Most strains are fairy aerotolerant while optimal growth is achieved under microaerophilic or anaerobic conditions. Increased CO_2 concentration (~5%) may stimulate growth. Most lactobacilli grow best at mesophilic temperature with an upper limit around 40°C [1]. Lactobacilli should be incubated in jars evacuated and filled with 90% N_2 or H_2 plus 10% CO_2 or in anaerobic jars using H_2 plus CO_2 generating kits.

1.3 Ecology of *Lactobacillus*

Lactobacilli grow under anaerobic conditions or at least under reduced oxygen tension in all habitats providing carbohydrates, proteins and nucleic acids and vitamins. A mesophilic to slightly thermophilic temperature range is favorable. Lactobacilli are generally acidophilic. They decrease the pH of their substrate by lactic acid formation to below pH 4.0, thus preventing growth of other competitors except other lactic acid bacteria and yeasts. These properties make lactobacilli valuable inhabitants of the intestinal tract of humans and animals and important contributors to food technology. Many species of *Lactobacillus* are microbiota of humans and animals.

Lactobacilli occur in nature in low numbers at all plant surfaces and together with other lactic acid bacteria, grow luxuriously in all decaying plant material, especially decaying fruits. Thus, lactobacilli are important for the production as well as the spoilage of fermented vegetable feed and food and beverages. Most species isolated have been: *L. plantarum, L. brevis, L. coryneformis, L. casei, L. curvatus, L. sake* and *L. fermentum* [53-56].

In milk and dairy products, milk contains no lactobacilli when it leaves the udder, but becomes very easily contaminated with lactobacilli by dust and dairy containers. After prolonged incubation bacteria take over, due to their higher acid tolerance [1]. *L. delbrueckii* subsp. *bulgaricus* is a component of the well known yogurt microbiota [57].

Lactobacilli play an important role during the processing of fermented sausages containing added sucrose. Various species of lactobacilli multiply during cold storage of meat products. This process delays spoilage by proteolytic bacteria.

The most common naturally occurring species found in ripening raw sausages are *L. plantarum*, *L. brevis*, *L. farciminis*, *L. alimentarius*, *L. sake* and *L. curvatus* [56, 58].

1.4 *Lactobacillus* of the human digestive tract

Lactobacillus species are one of the most commonly found gram-positive bacteria in the human microbiota. The different numbers and species of lactobacilli depend on the genetic background of host as well as their age and health [59]. Lactobacilli have been found in the oral cavity, gastrointestinal tract, vaginal and breast milk [1, 60, 61]. The *Lactobacillus* of gastrointestinal system consists of various species, subspecies and the most frequently occurring lactobacilli belong to six species: *L. acidophilus, L. salivarius, L. casei, L. plantarum, L. fermentum* and *L. brevis* [59]. In addition, the frequently occurrence of *L. reuteri* in the gastrointestinal tract of humans and animal has also been detected [1, 5, 62].

In the oral cavity, a wide range of *Lactobacillus* species have been found. These species are *L. acidophilus, L. salivarius, L. casei, L. rhamnosus, L. plantarum, L. fermentum, L. cellobiosus, L. buchneri*, and *L. brevis* [59].

Lactobacillus species could be detected in all parts of the human gastrointestinal tract including the stomach [62], which is characterized by a pH of 2.2–4.2. Relatively few bacterial species can tolerate these acidic conditions and most organisms ingested with food and saliva are killed by the hydrochloric acid, reducing the population to about 10^3 CFU/ml, containing mainly lactobacilli and streptococci [44, 63]. In the duodenum and jejunum, lactobacilli and enterococci are the dominant bacteria [44, 64, 65]. The microbiota becomes more complex in the ileum, being

qualitatively similar to that of the large intestine and the relative proportion of lactobacilli decreases. *Lactobacillus* species can be cultured from human feces at counts varying from none to $<10^9$ CFU/gm feces [64, 66, 67]. Tannock, et al. investigated the succession of lactobacilli in feces of 10 human subjects during a period of fifteen months. They found that the dominant and persistent species were *L. ruminis, L. salivarius*, *L. acidophilus*, *L. crispatus* and *L. gasseri* which were regularly detected in the feces [67]. *L. ruminis* was also detected as the predominant species over several months and *L. salivarius*, *L. acidophilus, L. crispatus* and *L. gasseri* could be detected regularly [68, 69]. *L. reuteri* has been rarely detected in human fecal samples in recent studies either by culture or by nucleic acid-based methods [70-71]. Furthermore, these studies indicated that lactobacilli such as *L. paracasei*, *L. rhamnosus*, *L. delbrueckii*, *L. brevis*, *L. johnsonii*, *L. plantarum* and *L. fermentum* are rather transient, persist for limited times, or in undetectable low numbers that may increase in response to dietary factors or changes in the host's conditions [62].

The presence of the lactobacilli in the digestive tract has historically been considered as beneficial to the host. At the beginning of the last century, Elie Metchnikoff (1845–1916) stated that toxic substances produced by members of the intestinal microbiota are absorbed from the intestinal tract and contribute to the aging process [71]. Microbes capable of degrading proteins, releasing ammonia, amines and indole were considered harmful and bacteria like lactobacilli (which ferment carbohydrates to obtain energy and have little proteolytic activity) were thought to be beneficial [72, 73]. Lactobacilli are considered to benefit the health of the consumer when ingested as probiotics [64, 74]. *Lactobacillus* species commonly

detected in the intestine (fecal samples), oral cavity and associated with food and probiotics products were shown in Table 1.

Table 1. *Lactobacillus* species commonly detected in the intestine (fecal samples), oral cavity and associated with food and probiotics products [68].

Species	Oral Cavity	Feces	Food	Probiotics
L. crispatus	+	+	-	+
L. gasseri	+	+	-	+
L. reuteri	-	+	+	+
L. ruminis	-	+	-	-
L. salivarius	+	+	-	+
L. acidophilus	+	+	-	+
L. brevis	+	+	+	-
L. casei	+	+	+	+
L. delbrueckii	-	+	+	+
L. fermentum	+	+	+	+
L. johnsonii	-	+	-	+
L. paracasei	+	+	+	+
L. plantarum	+	+	+	+
L. rhamnosus	+	+	+	+
L. sakei	+	-	+	-
L. curvatus	+	-	+	-

1.5 Antimicrobial compounds of *Lactobacillus*

The ability of lactic acid bacteria especially, *Lactobacillus* to produce the antimicrobial substances has historically long been used to preserve foods. They have ability to produce various antimicrobial substances which can be classified as low-molecular-mass (LMM) compounds such as organic acid (lactic acid, acetic acid and propionic acid), hydrogen peroxide (H_2O_2), diacetyl (2, 3-butanedione), uncharacterized compounds and high-molecular-mass(HMM) compounds like bacteriocins [75].

Organic acids

Lactic acid is produced by homofermentation or equimolar amounts of lactic acid, acetic acid, propionic acid, ethanol and carbon dioxide are produced by heterofermentation. Acetic acid is the strongest inhibitor and has a wide range of inhibitory activity, inhibiting yeasts, molds and bacteria [76] while propionic acid has been observed to exert a strong antimicrobial effect, in particular towards yeasts and molds [77]. Mixtures of lactic and acetic acids have been observed to reduce the growth rate of *S. enterica* ser. var. Typhimurium more than either acid alone, suggesting a synergistic activity [78].

Hydrogen peroxide

Hydrogen peroxide is produced in the presence of oxygen. The bactericidal effect of hydrogen peroxide (H_2O_2) has been attributed to its strong oxidizing effect on the bacterial cell. Also, some of the hydrogen peroxide producing reactions scavenges oxygen, thereby creating an anaerobic environment that is unfavorable for

certain organisms. It has been suggested that hydrogen peroxide production is particularly important for colonization of the urogenital tract by lactobacilli. Colonization by such lactobacilli has been found to decrease the acquisition of human immune deficiency virus (HIV) infection, gonorrhea and urinary tract infection [79].

Diacetyl

Diacetyl (2, 3-butanedione) is produced by citrate fermentation. It was identified as the aroma and flavor component in butter [80]. The antimicrobial activity of diacetyl has been documented since 1927 and was reviewed by Jay, J.M. [80]. This author found that this molecule is characterized by a broad antimicrobial activity at concentrations ranging between 200 and 1000 part per million (ppm). Gram-positive bacteria were more resistant, while gram-negative and yeasts exhibited a higher sensitivity to this molecule [80]. Diacetyl was able to strongly inhibit *Escherichia coli* O157:H7 and *Salmonella* Typhimurium at concentrations 50 ppm [81].

Reuterin

Reuterin, a low–molecular-mass compound, is produced by *L. reuteri*, a heterofermentative species and a member of microbiota of gastrointestinal tract of humans and animals. It is formed during the anaerobic growth on a mixture of glucose and glycerol by the action of glycerol dehydratase which catalyzes the conversion of glycerol into reuterin, 3-hydroxypropanal [27]. During log phase, no reuterin is produced since it is reduced by the reducing power from glucose metabolism. However, when cells enter stationary phase, reuterin starts to accumulate [82]. Although other bacteria also assimilate glycerol via the same pathway, accumulation

and excretion of reuterin appears to be a specific property of *L. reuteri* [83, 84]. Reuterin has a very broad spectrum of antimicrobial activity. It was found to have antibacterial, antifungal and antiprotozoal activity. Harmful organisms sensitive to reuterin include species of *Salmonella, Shigella, Vibrio,* enterohemorrhagic *E. coli*, enterotoxigenic *E. coli*, *Clostridium, Staphylococcus, Listeria, Candida* and *Trypanosoma* [85-88].

Bacteriocins

Bacteriocins are compounds produced by bacteria in order to inhibit the growth of other bacteria. Bacteriocins can be regarded as antibiotics, but their mode of action is different from many antibiotics. They have a narrow killing spectrum and thus they are generally able to kill only bacteria closely related to the producing strains [89].

Bacteriocins produced by *Lactobacillus* can be divided into four major classes: Class I-lantibiotics or bacteriocins which are small peptides (<5 kDa), containing unusual amino acids not normally found in nature; Class II-small hydrophobic bacteriocins which are heat-stable peptides (<13 kDa); Class III-large bacteriocins which are heat-labile proteins (>30 kDa); and Class IV-complex bacteriocins which are proteins with lipid and/or carbohydrate moieties. Class I and II bacteriocins are currently the main classes of bacteriocins due to their abundance and potential use in commercial applications [75]. Most of the bacteriocins produced by *Lactobacillus* spp. belong to the Class II bacteriocins. *L. plantarum* is most often associated with bactericin production. A number of bacteriocins have been isolated from various *L. plantarum* strains [90].

1.6 Methods for evaluation of antimicrobial activity

The agar diffusion method

Agar diffusion method has long been used for testing antimicrobial activity and is probably the most commonly used for detection of antimicrobial activity [75]. The method has been widely used for biologically derived compounds. It includes agar well diffusion assay [91] and disc assay [92]. In this test, an antimicrobial compound is applied to an agar plate on a paper disc or a well [92]. The compound diffuses into agar resulting in a concentration gradient that is inversely proportional to the distance from the disc or well. The size of inhibition zone around the disc or well is a measure of the degree of inhibition. The incubation conditions are dependent on the indicator organisms used. Since highly hydrophobic antimicrobial compounds cannot diffuse in agar, they are not suitable for tests by this method [75].

Several modified procedures based on the agar diffusion method have also been used for testing antimicrobial activity. These procedures include the agar spot method [93] and spot-on-lawn method [94].

The agar and broth dilution methods

Agar and broth dilution methods are suitable for microorganisms with variable growth rate and for anaerobic or microaerophilic microorganisms. The results are expressed as minimum inhibitory concentration (MIC), which is the lowest concentration of an antimicrobial that prevent growth of a microorganisms after a specific incubation period. In this method, an antimicrobial agent is serially diluted and a single concentration added to a culture tube or plate with nonselective broth or

melted agar medium, which is then inoculated with test organisms and incubated. The MIC is defined as the lowest concentration at which no growth occurs or absence of turbidity in a medium following incubation. The broth dilution assay has been used for the detection of the antimicrobial activity of reuterin produced by *L. reuteri* and the activity of reuterin was expressed as MIC values or the maximum dilutions of the reuterin fraction [27, 85].

New rapid screening methods for the detection of antimicrobial activity have been developed. In one method, indicator organisms are exposed to bacteriocins after staining with carboxyfluorescein diacetate [95]. Fluorescence is measured by flow cytometry and the effect of bacteriocin is seen as a decrease of fluorescence when the fluorescent compound leaks from the cells. Another method has used bioluminescent indicator strains in screening of antimicrobial activity. Luciferase genes are transformed into indicator strains and indicator strains start to produce light in reaction [96]. This method increases the sensitivity and allows for real-time assessment of antimicrobial activity [75].

2. *Lactobacillus* as Probiotics

2.1 Background of probiotics

The first significant introduction of the probiotic concept was by Nobel Prize Laureate, Elie Metchnikoff, at the beginning of the 1900s. He believed that the complex microbial population in the colon was adversely affecting the host through so-called 'autointoxication', and reported that Bulgarian peasants, who consumed large quantities of fermented milk containing lactic acid bacteria were associated with good health and longevity [72]. The milk contained the microorganism "Bulgarican bacillus" which was later renamed *Lactobacillus bulgaricus*. Metchnikoff reasoned that these bacteria eliminated putrefactive bacteria from the gastrointestinal tract [97]. The works of Metchnikoff are regarded as the birth of probiotics [98]. This was attributed to the health-promoting values of the live organisms [90]. Subsequent research looked to confirm that the consumption of lactic acid bacteria was having a beneficial effect on health. In Japan, Shirota selected beneficial strains of lactic acid bacteria which could survive passage through the intestine, and subsequently used them to develop fermented milk drinks, known as *L. casei* Shirota in Yakult product [99]. It was soon established that there were many species of lactic acid bacteria in the intestine and these have subsequently been incorporated into many probiotic preparations [90]. Lactobacilli and bifidobacteria are the most frequently used genera as probiotics [100]. At present, probiotics products are available in variety of forms, including dairy foods, fermented milk, food supplements and dietary supplements and the range of products continued to expand. In parallel, the market for such foods

continues to develop, with most activity in developed countries, in particular in Europe, Japan and the United States [101].

2.2 Definition of probiotics

The term "probiotics" which comes from the Greek meaning "for life" was first used to described substances produced by one microorganism that stimulate the growth of another microorganism [102]. Fuller defined a probiotics as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" [103]. Fuller's definition has since been broadened to state that "a probiotic is a mono-or mixed culture of live microorganisms which, when applied to animal or man, affect the host beneficially by improving the properties of the indigenous microflora" [104]. Salminen et al. [105] proposed that probiotics be defined as microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host. A recent formal definition of probiotics was agreed by a working party of European scientists and is given as "a live microbial feed supplement that is beneficial to health" [106]. This emphasized the importance of definitive improvements in health. A probiotic effect can therefore be manifested via the gut microflora by ingestion of viable micro-organisms, either in the form of specific preparations such as powders, tablets or capsules, or through yoghurts and other fermented foods. They can contain only one, or several different species of microorganisms [90].

2.3 Probiotics properties

For the selection and assessment of potential probiotics, several research groups have recommended that a microorganism should have some predefined criteria in order to be considered as probiotics. These criteria were summarized in Table 2 [100, 107]. Strains of human origin are most suitable because some health promoting benefits may be species specific and microorganisms may perform optimally in the species from which they were isolated [108]. However, it is the specificity of the action, not the source of the microorganism that is recognized as being most important when selecting probiotics strains for particular applications [109]. All probiotic strains should have generally recognized as safe (GRAS) status. To survive passage through the stomach and small intestine, probiotic strains must tolerate the acidic and protease-rich conditions of the stomach, and survive and grow in the presence of bile and acids. Adherent probiotics strains are desirable because they have a greater chance of becoming established in the gastrointestinal tract, thus enhancing their probiotics effect [109]. The production of antimicrobial substances is regarded as important selection criteria for probiotics. Many probiotic *Lactobacillus* species have been shown to produce antimicrobial substances [110]. Ability to modulate immune responses is important for probiotic properties to modulate immune responses in immune dysfunction state. Probiotic microorganisms should also be technologically suitable for incorporation into food products and should be capable of surviving industrial applications [107, 111].

Table 2. Criteria for an ideal probiotic strain [100, 107]

Desirable characteristics of an ideal probiotic microorganisms
Human origin
Generally recognized as safe (GRAS) status
Resistance to gastric acidity and bile toxicity
Adherence to gut epithelial tissue
Ability to colonize the gastrointestinal tract
Production of antimicrobial substances
Ability to modulate immune responses
Amenable to large scale fermentation and commercial production

2.4 Mechanism of action of probiotics

There are many proposed mechanisms by which probiotics may protect the host from intestinal disorders, but the main mechanisms are not fully elucidated. Much work remains to clarify the mechanisms of action of particular probiotics against particular pathogens. In addition, the same probiotic may inhibit different pathogens by different mechanisms [16]. Listed below is a brief description of mechanisms by which probiotics may protect the host against intestinal disease [16, 90].

Production of inhibitory substances

Probiotic bacteria produce a variety of substances that are inhibitory to both gram-positive and gram-negative bacteria. These inhibitory substances include organic acids, hydrogen peroxide, diacetyl, reuterin and bacteriocins as described above. These compounds may reduce not only the number of viable cells but may also affect bacterial metabolism or toxin production [16].

Blocking of adhesion sites

Competitive inhibition for bacterial adhesion sites on intestinal epithelial surfaces is another mechanism of action for probiotics [112-114]. Consequently, some probiotic strains have been chosen for their ability to adhere to epithelial cells. In this way, they may resist peristalsis which would otherwise flush them from the gut. As well as occupying a niche at the expense of potentially harmful organisms, they may specifically block the adherence of enteropathogens [16, 90].

Competition for nutrients

The ability to compete for limiting nutrients is an important factor that determines composition of the gut microbiota, with species that are unable to compete being effectively eliminated from the system. Bacteria in the large intestine are subject to a range of substrate availability; species in the proximal colon have a large supply of nutrients, provided by dietary residues transiting from the small intestine, while those occupying the distal region of the colon have more limited substrate availability. Increasing lactobacilli numbers by way of a probiotic may thereby

decrease the substrate available for other bacterial populations. However, the evidence that this occurs *in vivo* is lacking [16, 90].

Degradation of toxin receptor

The postulated mechanism by which *Saccharomyces boulardii* protects animals against *C. difficile* intestinal disease is through degradation of the toxin receptor on the intestinal mucosa [115, 116].

Stimulation of immunity

Recent evidence suggests that stimulation of specific and nonspecific immunity may be another mechanism by which probiotics can protect against intestinal disease [117].

2.5 Strains used as probiotics

Many microorganisms have been used or considered for use as probiotics [16]. A probiotic preparation may contain one or several different strains of microorganisms. Because viable and biologically active microorganisms are usually required at the target site in the host, it is essential that the probiotics be able to withstand the host's natural barriers against ingested bacteria. The most commonly used probiotics are strains of lactic acid bacteria especially, *Lactobacillus* and *Bifidobacterium* [16]. The beneficial effects of *Lactobacillus* and *Bifidobacterium* have been discussed for decades. Bacteria in these two genera resist gastric acid, bile salts and pancreatic enzymes, adhere to intestinal mucosa and readily colonize the intestinal tract. They are considered important components of the gastrointestinal

microbiota and are relatively harmless. *Lactobacillus* species are typically used as human probiotics because they are easy to cultivate in bulk and have a long history of safe use in fermented foods [118]. As shown in Table 3, *Lactobacillus, Bifidobacterium* and other microorganisms are currently being used as probiotics either singly or in combination. *Lactobacillus* species have been demonstrated to inhibit the *in vitro* growth of many enteric pathogens including *E. coli* O157:H7, *S.* Typhimurium, *Staphylococcus aureus*, *Campylobacter jejuni, Clostridium perfringens* and *C.difficile* [36, 119-123]. Therefore, they have been used in both humans and animals to treat a broad range of gastrointestinal disorders [122-123]. Hundreds of publications have described the use of probiotics to prevent and treat a variety of gastrointestinal disorders.

Table 3. Microorganisms considered as probiotics [100]

Lactobacillus species	*Bifidobacterium* species	Others
L. acidophilus	*B. bifidum animalis*	*Bacillus cereus*
L. rhamnosus	*B. longum*	*Clostridium butyricum*
L. gasseri	*B. breve*	*Escherichia coli*
L. casei	"*B. infantis*"	*Proprionibacterium*
L. reuteri	*B. lactis*	*Freundendsreichii*
L. bulgaricus	*B. adolescentis*	"*Saccharomyces boulardii*"
L. plantarum		*Enterococcus feecalis*
L. johnsonii		*Streptococcus thermophilus*
		Lactococcus species
		VSL#3 (*L.bulgaricus, L. plantarum, B. longum, B. infantis, B. breve, S. salivarius* subsp. *thermophilus*)

3. Role of probiotics in intestinal disorders and infectious diarrhea

Antibiotic-induced diarrheal disease

Diarrhea is the most common side effect of antimicrobial therapy with 20% of patients receiving an antibiotic developing this condition [124]. The pathogenesis of antibiotic-induced diarrhea is not understood but is undoubtedly related to quantitative and qualitative changes in the intestinal microbiota [125]. Many of the studies that have attempted to demonstrate the usefulness of probiotics in antibiotic-associated diarrhea have used it prophylactically. However, because of the low incidence of antibiotic-associated diarrhea and the variable intensity of the diarrhea, it is not practical from a cost-benefit viewpoint to treat all patients receiving antibiotic therapy in this way with a probiotic. Furthermore, it is not possible to predict which patient will develop antibiotic-associated diarrhea. Nonetheless, several probiotics have been used in an attempt to prevent antibiotic-associated diarrhea [16]. Adam et al. [34] prospectively treated 388 ambulatory patients, receiving either tetracycline or a β-lactam, concurrently with placebo or "*Saccharomyces boulardii*". The incidence of diarrhea in patients receiving the placebo was 17.5%, whereas in patients receiving "*S. boulardii*", it was 4.5%. These results were confirmed in another study of 193 patients receiving at least one broad-spectrum β-lactam antibiotic [126]. Of the 97 patients receiving *S. boulardii*, only 7.2% developed antibiotic-associated diarrhea compared with 14.6% of the 96 patients receiving placebo. Lactinex, a commercial preparation containing *L .acidophilus* and *L. bulgaricus*, was used in a placebo-controlled study of 79 hospitalized patients receiving ampicillin [127]. The rationale for using *Lactobacillus* in these patients is based on the observation that antibiotic

therapy often causes a loss or reduction in the number of intestinal *Lactobacillus*. Thirty-six patients received concurrent Lactinex and 43 patients received placebo. None of the patients receiving Lactinex developed ampicillin-induced diarrhea, whereas 14% of the placebo group developed diarrhea.

Clostridium difficile-associated intestinal disease

Clostridium difficile is a classic example of the opportunistic proliferation of an intestinal pathogen after breakdown of colonization resistance due to antibiotic administration. After antibiotic intake by animals and humans, *C. difficile* colonizes the intestine and releases two protein exotoxins, toxin A and toxin B, which mediate the diarrhea and colitis caused by this microbe. Toxigenic *C. difficile* is the cause of 20–40% of cases of antibiotic- associated diarrhea [128-130]. The multiple relapses can occur and the relapses can be more severe than the original disease. The mechanism of relapse is unknown but is probably due to the survival of *C. difficile* spores in the intestinal tract until the antibiotic is discontinued [131]. An attractive alternative to antibiotic therapy is to use probiotics to restore intestinal homeostasis. *L. paracasei, L. plantarum* and *L. salivarius* have been reported to inhibit several *C. difficile* toxin A-producing strains *in vitro* [121, 30]. In a placebo-controlled study, McFarland et al. [132] examined standard antibiotic therapy with concurrent *S. boulardii* or placebo in 124 adult patients, 64 patients with an initial episode of *C. difficile* disease and 60 patients with a history of at least one prior episode of *C. difficile* disease. The investigators found that in patients with an initial episode of *C. difficile*, there was no significant difference in the recurrence of *C. difficile* disease in the placebo or *S. boulardii* groups. However, in patients with prior *C. difficile* disease, *S. boulardii*

significantly inhibited further recurrences of disease. The investigators concluded that in combination with standard antibiotics, *S. boulardii* is an effective and safe therapy for patients with recurrent *C. difficile* [16].

Rotavirus diarrhea

Rotavirus is a common cause of infantile diarrhea. Rotavirus is a significant cause of infant morbidity and mortality, particularly in developing countries [133, 134]. The principal means of treatment is oral rehydration although an effective vaccine that should decrease dramatically the health impact of rotavirus infections has recently become available. *Lactobacillus* has demonstrated some promise as a treatment for rotavirus infection [135, 136]. Isolauri [137] studied the children with diarrhea treated with either *Lactobacillus* GG or placebo. Approximately 80% of the children with diarrhea were positive for rotavirus. The investigators demonstrated that the duration of diarrhea was significantly shortened in patients receiving *Lactobacillus* GG and the effect was even more significant when only the rotavirus-positive patients were analyzed.

Traveler's diarrhea

The incidence of diarrhea in travelers to foreign countries varies from 20 to 50% depending on the origin and the destination of the traveler, as well as the mode of travel. Although various infectious agents can cause traveler's diarrhea, enterotoxigenic *E. coli* is the most common. Several probiotics have been examined for their ability to prevent traveler's diarrhea, including *Lactobacillus, Bifidobacterium*, *Streptococcus* and *Saccharomyces* [138-140]. These studies have involved several different groups of travelers such as Finnish travelers to Turkey,

American travelers to Mexico, British soldiers to Belize and European travelers to Egypt. The results from these studies have been extremely variable. For example, in the study of Finnish travelers to Turkey, the travelers had two different destinations [139]. In one destination, *Lactobacillus* GG provided protection against traveler's diarrhea but failed to protect travelers at the other destination. Different etiologic agents may have involved in these two locations, but this possibility was not examined.

Food borne pathogen-associated gastrointestinal infections

Disruption of the normal balance of the resident gastrointestinal microbiota can allow establishment and growth of transient enteropathogens like *Salmonella, Campylobacter, E. coli, Listeria* and *Shigella* spp. Several *Lactobacillus* species have been examined and exhibited antagonistic to *B. cereus, E. coli, S. aureus, Yersinia enterocolitica* and *Listeria in vitro* [120]. In addition, *Lactobacillus* sp., *L. acidophilus, L. plantarum* and *L. brevis* have been shown to inhibit *C. jejuni*, *E. coli* O157:H7 and *S.* Typhimurium [30, 119]. Studies using animal models have established the ability of certain probiotics to inhibit pathogen growth [90].

***Helicobacter pylori* gastroenteritis**

Helicobacter pylori has been shown to be an important etiologic agent of chronic gastritis as well as gastric and duodenal ulcers. It has also been postulated that chronic *H. pylori* infection leads to stomach carcinoma. *L. acidophilus* and *L. rhamnosus* confer inhibitory effects on *H. pylori in vitro* [141, 142]. With an animal model using gnotobiotic mice, it was found that *L. salivarius* was effective in

inhibiting *H. pylori* [143], while in a human trial, *L. acidophilus* was effective at inhibiting colonization of the organism [144].

4. Role of Probiotics in Inflammatory Bowel Diseases

Inflammatory bowel diseases (IBD) including two forms of Crohn's disease and ulcerative colitis, is a significantly public health in Western societies and their etiologies remain unclear. IBD is characterized clinically by chronic inflammation in the large and/or small intestine. The most common clinical manifestation of ulcerative colitis is an inflammation of the colon. No specific treatment is available for either disease [16]. Evidence suggests that abnormal activation of the mucosal immune system against the enteric microbiota is the key event triggering inflammatory mechanisms that induce mucosal injury and intestinal lesions to chronicity. Patient showed an increased mucosal secretion of IgG antibodies against commensal bacteria [145] and mucosal T-lymphocytes are hyperreactive against antigens of the commensal microbiota, suggesting that local tolerance mechanisms are abrogated [146]. Evidences suggested that TNF-α, quantities in serum, stool and intestinal tissues are elevated in patients with Crohn's disease [147, 40] and imbalance in TNF-α and TNF-α inhibitors plays an important role in gut inflammation in patients with IBD [109]. For instance, TNF-α could induce epithelial cells to secrete IL-8, and express membrane Toll-like receptor 4 (TLR4) excessively [108, 109]. TLR4 could enable intestinal epithelia hyperreactive in response to lipopolysaccharides (LPS), the component of gram-negative bacteria cell walls, and IL-8 has chemotactic and stimulatory properties [42]. As a result, inflammatory cells infiltrate and the inflammatory reaction is therefore increased.

However, some *Lactobacillus* strains including *L. casei* suppress the spontaneous release of TNF-α by inflamed tissue and also the inflammatory response induced by *E. coli* [148, 149]. Probiotics have been tested in animal models of bowel inflammation. In IL-10 deficient mice which spontaneously develop colitis, oral administration of either VSL#3, a mixture of eight bacterial strains, or *L. plantarum* significantly decreased histological colitis scores in this animal model [150, 151]. Combination of *L. paracasei* and *L. reuteri* reduced intestinal inflammation in *H. hepaticus*-challenged IL-10 deficient mice and the levels of pro-inflammatory colonic cytokine, TNF-α, were lowered in *Lactobacillus*-treated mice [47]. In addition, administration of *L. reuteri*, TNF-α inhibitory strain, to IL-10 deficient mice resulted in decrease in colitis in treated animals [48]. *L. fermentum* and *L. lactis* have been shown to reduce colitis in mouse models [152, 153]. Treatment with anti-TNF-α antibody is effective in cases of intractable Crohn's disease [154]. Some researchers found that manipulating the normal intestinal microbiota using probiotics had a beneficial effect on health by altering the microbial environment, and some components of the microbiota could down-regulate inflammation when supplemented to patients with gastrointestinal diseases [155, 156]. A pilot study [155] was conducted to investigate the possible effects of *Lactobacillus* GG in four children with active Crohn's disease. Three patients treated with oral *Lactobacillus* GG showed significant improvement in terms of clinical outcome, and it was possible to taper the dose of corticosteroids. *Lactobacillus* GG seems to be effective in improving the clinical status of children with Crohn's disease. Gionchetti [157] had evaluated the efficacy of a probiotic preparation VSL#3 in maintenance of remission in chronic pouchitis compared with placebo. VSL#3 contains viable lyophilized bacteria of four strains of

lactobacilli, three strains of bifidobacteria, and one strain of *Streptococcus salivarius* subsp. *thermophilus*. Forty patients in clinical and endoscopic remission were randomized to receive either VSL#3 or an identical placebo for nine months. Three patients (15%) in the VSL#3 group had relapses within the nine month follow up period, compared with 20 (100%) in the placebo group. Fecal concentrations of lactobacilli, bifidobacteria, and *S. thermophilus* increased significantly from baseline levels only in the VSL#3 treated group. These results suggested that oral administration of probiotic preparation was effective in preventing flare-ups of chronic pouchitis.