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Modern Approaches in Probiotics Research to Control Foodborne Pathogens

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Abstract

Foodborne illness is a serious public health concern. There are over 200 known microbial, chemical, and physical agents that are known to cause foodborne illness. Efforts are made for improved detection, control and prevention of foodborne pathogen in food, and pathogen associated diseases in the host. Several commonly used approaches to control foodborne pathogens include antibiotics, natural antimicrobials, bacteriophages, bacteriocins, ionizing radiations, and heat. In addition, probiotics offer a potential intervention strategy for the prevention and control of foodborne infections.

This review focuses on the use of probiotics and bioengineered probiotics to control foodborne pathogens, their antimicrobial actions, and their delivery strategies. Although probiotics have been demonstrated to be effective in antagonizing foodborne pathogens, challenges exist in the characterization and elucidation of underlying molecular mechanisms of action and in the development of potential delivery strategies that could maintain the viability and functionality of the probiotic in the target organ.



1. INTRODUCTION

Foodborne illness is a serious public health concern. The global burden of foodborne illness is currently unknown. However, the World Health Organization (WHO) reported that in 2005, 1.8 million people died from diarrheal diseases, largely due to contaminated food and water (Greig & Ravel, 2009; Newell et al., 2010). In the United States, the Centers for Diseases Control and Prevention (CDC) estimates that each year there are about 48 million cases of foodborne infections with 128,000 hospitalizations and 3000 deaths (Scallan et al., 2011). There are over 200 known microbial, chemical, or physical agents that can result in illness when consumed (Newell et al., 2010). Of these, microbial source comprising of bacterial, viral, and fungal is of major concern. CDC estimates that of all the foodborne infections, 44% of the hospitalizations and deaths are attributed to 31 known pathogens (Scallan et al., 2011). In light of this serious public health crisis, efforts have been directed toward the detection, control, and prevention of well-recognized foodborne pathogens and diseases in the food chain. It is estimated that a reduction in foodborne illness by 10% would keep about 5 million Americans from getting sick each year (Scallan et al., 2011). With increasing trend in consumer preference for safe and wholesome food, probiotics offer an effective and alternative intervention strategy to control foodborne illnesses.

1.1. Overview of foodborne pathogens and diseases

The etiologic agents for foodborne infections comprise bacterial, viral, parasitic, and fungal (Table 5.1). These pathogens have the potential to cause significant morbidity or mortality, and have low infective dose, high virulence potential, ubiquitous in nature, and are stable in food products.

Of all the pathogens listed in Table 5.1, CDC estimates that the majority of the illnesses, hospitalizations, and deaths are caused by five known

Table 5.1 List of major foodborne microbial pathogens

Bacterial	Viral	Parasitic	Fungal
<i>Aeromonas hydrophila</i>	Aichivirus	<i>Cryptosporidium parvum</i>	<i>Alexandrium tamarense</i>
<i>Arcobacter butzleri</i>	Astrovirus	<i>Cyclospora cayatenesis</i>	<i>Aspergillus</i> spp.
<i>Bacillus cereus/subtilis/licheniformis</i>	Calcivirus (Norovirus)	<i>Entamoeba histolytica</i>	<i>Fusarium</i> spp.
<i>Brucella abortus/melitensis/suis</i>	Hepatitis A virus	<i>Giardia intestinalis/lambliia</i>	<i>Microcystis aeruginosa</i>
<i>Campylobacter jejuni/coli</i>	Hepatitis E virus	<i>Isospora belli</i>	<i>Penicillium</i> spp.
<i>Clostridium botulinum</i>	Rotavirus	<i>Taenia saginata/solium</i>	
<i>Clostridium perfringens</i>		<i>Toxoplasma gondii</i>	
<i>Cronobacter sakazakii/malonaticus/turicensis</i>		<i>Trichinella spiralis</i>	
<i>Escherichia coli</i> (pathogenic)			
<i>Listeria monocytogenes</i>			
<i>Mycobacterium paratuberculosis</i>			
<i>Plesiomonas</i> spp.			
<i>Salmonella enterica</i>			
<i>Shigella</i> spp.			
<i>Staphylococcus aureus</i>			
<i>Vibrio cholera/parahaemolyticus/vulnificus/fluvialis</i>			
<i>Yersinia enterocolitica</i>			

pathogens, which include Norovirus, nontyphoidal *Salmonella*, *Clostridium perfringens*, *Campylobacter* spp., and *Staphylococcus aureus*.

These pathogens enter into the food system through contaminated raw materials, water, humans, meat animals, wild life, and insect vectors. There are several factors that affect the trends in the occurrence of foodborne illness: large-scale production and wide distribution of food, globalization

of food supply, eating outside of the home, microbial genomic diversification yielding the emergence of new pathogens, and growing population of at-risk consumers. The diseases caused by these pathogens have different consequences and sequel (Table 5.2).

1.2. Various strategies to control pathogens

Foodborne pathogens sicken more than 48 million Americans annually, that is, 1 in 7 in the U.S. population (Scallan et al., 2011). Reducing foodborne illnesses by 10% would keep about 5 million Americans from getting sick every year. Food safety concerns are also elevated because of consumers demand for high quality, low preservatives, and minimally processed convenient ready-to-eat meals. Such foods are highly vulnerable to contamination and heighten public health safety concerns. This has led to the development of several control strategies both by the government and the industry. Strategies to control foodborne infections can be classified as preharvest and postharvest interventions. Traditionally, much of the research effort was aimed at improving the safety of meat products as postslaughter sanitation and product formulations. However, the continual incidence of outbreaks and increase in knowledge about the pathogens have led to the development of preharvest intervention strategies (Doyle & Erickson, 2012). Preharvest intervention step is a logical food safety approach that allows reduced levels of pathogen loads in the incoming raw materials (Callaway, Anderson, et al., 2003; Soon, Chadd, & Baines, 2011).

Antibiotics are known to alter the microbiological ecology of the intestinal tract (Callaway, Edrington, et al., 2003). This led to the prophylactic use of antibiotics in animal agriculture to control disease and improve animal growth rate and efficiency. With emergence of antibiotic resistance among pathogens, research has focused on the use of naturally occurring antimicrobials as an alternative to control foodborne pathogens in live animals and foods. Antimicrobials comprise organic acids, essential oils, plant extracts, bacteriocins, probiotics, and bacteriophages (Baugher & Klaenhammer, 2011; Callaway, Anderson, et al., 2003; Hassan, Kjos, Nes, Diep, & Lotfipour, 2012; Negi, 2012). Chemical rinses using organic acids that are generally recognized as safe such as acetic, lactic, and citric acids are commonly used in the meat industry to rinse animal carcasses and produce (fruits and vegetables) (Sirsat, Muthaiyan, & Ricke, 2009). These acids reduce the pH of the food and hence control the growth of microorganisms. Lactic acid is most effective when applied at higher temperatures and at a concentration of 2–4% (Sirsat et al., 2009).

Table 5.2 List of common foodborne pathogens, incubation period, symptoms, and possible food sources

Foodborne pathogens	Possible food source	Incubation period	Symptoms
<i>Bacillus cereus</i>	Meats, milk, rice, potatoes, pasta, vegetables, and cheese	30 min to 15 h	Diarrhea, abdominal cramps, nausea, and vomiting
<i>Campylobacter jejuni</i>	Raw milk, eggs, poultry, raw beef, water, cake icing	1–7 days	Nausea, abdominal cramps, diarrhea, headache
<i>Clostridium botulinum</i>	Low-acid canned foods, meats, sausage, fish	12–36 h	Nausea, vomiting, dry mouth, diarrhea, fatigue, headache, double vision, slurred speech, respiratory distress, flaccid paralysis
<i>Clostridium perfringens</i>	Undercooked meats, roast beef, and gravies	8–24 h	Abdominal cramps, diarrhea, dehydration
<i>Cryptosporidium parvum</i>	Contaminated water or milk, person-to-person transmission, raw or undercooked food	2–10 days	Watery diarrhea accompanied by mild stomach cramping, nausea, loss of appetite
<i>Escherichia coli</i> O157:H7 and Shiga toxin producing <i>E. coli</i> (STEC)	Ground beef, raw milk, undercooked beef, apple, green leafy vegetables	2–4 days	Hemorrhagic colitis, hemolytic uremic syndrome
<i>Giardia lamblia</i>	Contaminated soil, water, food, or surfaces	1–2 weeks	Diarrhea, loose or watery stool, stomach cramps, and lactose intolerance
Hepatitis A	Water, fruits, vegetables, iced drinks, shellfish, and salads	4–6 weeks	Fever, malaise, nausea, abdominal discomfort, hepatitis, jaundice
<i>Listeria monocytogenes</i>	Contaminated vegetables, milk, cheese, meat, sea food, smoked fish, ready-to-eat foods	2 days to 3 weeks	Meningitis, septicemia, miscarriage, stillbirth, neonatal listeriosis

Continued

Table 5.2 List of common foodborne pathogens, incubation period, symptoms, and possible food sources—cont'd

Foodborne pathogens	Possible food source	Incubation period	Symptoms
Norwalk, Norwalk-like, or Norovirus	Raw oysters, shellfish, water and ice, salads, frosting, person-to-person contact	12–60 h	Nausea, vomiting, diarrhea, abdominal cramps
Nontyphoidal <i>Salmonella</i> serovars	Meat, poultry, eggs, milk products	12–24 h	Nausea, diarrhea, abdominal pain, fever, headache, chills, prostration
<i>Staphylococcus aureus</i>	Custard or cream-filled baked goods, ham, poultry dressing, gravy, eggs, potato salad, cream sauces, sandwich fillings	1–6 h	Severe vomiting, diarrhea, abdominal cramping
<i>Shigella</i> spp.	Salads, raw vegetables, dairy products, poultry	12–50 h	Abdominal pain, cramps, fever, vomiting
<i>Toxoplasma gondii</i>	Domestic cat, bird or rodent feces, raw or undercooked food	5–23 days	Swollen lymph glands, fever, headache, muscle aches, abortion in pregnant women. Severe infection in immunocompromised people and unborn babies
<i>Vibrio parahaemolyticus</i> / <i>vulnificus</i>	Fish, shellfish, oysters	4 h to 4 days	Diarrhea, abdominal cramps, nausea, vomiting, headache, fever, and chills
<i>Yersinia</i> spp.	Raw milk, chocolate milk, water, pork, raw meats	1–3 days	Enterocolitis, may mimic appendicitis

These organic acids are sometimes used in combination with oxidizing agents such as hydrogen peroxide to enhance their antimicrobial efficacies. Essential oils extracted from clove, cinnamon, thyme, and oregano, and their components have been used in the control of foodborne pathogens such as nontyphoidal *Salmonella* (Johny, Hoagland, & Venkitanarayanan, 2010), *Escherichia coli* O157:H7 (Amalaradjou et al., 2010), and *Listeria monocytogenes* in microbiological growth media, on live animals or food systems (Hyldgaard, Mygind, & Meyer, 2012). Unlike antibiotics, essential oils have multifaceted antimicrobial effects thereby making it difficult for the bacteria to develop resistance.

Microbial contamination can also be controlled by the use of microbicidal treatments, such as ionizing radiations, and heating. Application of nonthermal methods such as high hydrostatic pressure, high-intensity pulsed electric fields, oscillating magnetic fields, intense light pulse, photosensitization, or a combination of above (hurdle approach) has also been shown to be effective (Lukšienė & Zukauskas, 2009; Morris, Brody, & Wicker, 2007). One of the most common physical methods of decontamination is irradiation (Radomyski, Murano, Olson, & Murano, 1994; Smith & Pillai, 2004). Food irradiation destroys the indigenous flora and prolongs shelf life of products during storage. Food is exposed to doses of ionizing radiation sufficient enough to create positive and negative charges to kill bacteria in the food. The type of physical method used and the dosage of the treatment depend on the type of food matrix to be decontaminated.

Biological methods of control include the use of bacteriophages, bacteriocins, and probiotics. In food matrix, these components can be present naturally or added extrinsically. These biological agents can be used at the pre- and postharvest phase to prevent bacterial contamination (Hagens & Loessner, 2007). Bacteriophages are viruses that can infect and kill bacteria and are considered alternatives to antimicrobials for use in the food industry (García, Martínez, Obeso, & Rodríguez, 2008) and for therapeutic application to treat diseases (Fischetti, 2008, 2010; Hanlon, 2007). However, bacteriophages have narrow target spectra, and some can be used only against a particular strain. This high degree of specificity allows phages to be used against targeted microorganisms in a mixed population without affecting the microbial ecosystem (Callaway, Anderson, et al., 2003). Bacteriophages have been used to control foodborne pathogens in farm animals against specific pathogens (Hagens & Loessner, 2010; LeJeune & Wetzel, 2007; Wall, Zhang, Rostagno, & Ebner, 2010). In addition, several phages or phage cocktails have been approved by the FDA for use

as food additive in ready-to-eat meat or for application in cattle/poultry prior to slaughter (Hagens & Loessner, 2010).

The use of probiotics, prebiotics, and synbiotics (combination of prebiotics and probiotics) has also gained increased attention in recent years. The use of microflora to reduce pathogen load in the gut is termed as a probiotic strategy (Callaway, Anderson, et al., 2003). Probiotic techniques involve the introduction of a normal microbial population into the gut to provide a nutrient (prebiotic) that is limiting and allows the growth of a specific subset of the gut microflora. The goal of this approach is to fill all the niches available in the gut so as to exclude the establishment of pathogenic microbes (Doyle & Erickson, 2006; Gaggia, Mattarelli, & Biavati, 2010; Patterson & Burkholder, 2003). Due to the increased concern about the emergence in antibiotic resistance, use of probiotics provides an effective alternative to combat foodborne illnesses (Baugher & Klaenhammer, 2011; Dobson, Cotter, Ross, & Hill, 2012; Hassan et al., 2012).

In addition, vaccine and antibody therapy also offer a viable option to reduce the burden of foodborne illness. Vaccine therapy involves the stimulation of the animal's immune system to limit pathogen colonization. Two types of vaccines can be employed to immunize food animals: the use of killed or inactivated bacterial cells or live attenuated cells. This approach has been used in poultry to reduce the colonization of *Salmonella* using *Salmonella*-specific antibodies (De Buck, Van Immerseel, Haesebrouck, & Ducatelle, 2005; Tellez et al., 2001). Similar approach in cattle and swine has shown promising results by enhancing immunoglobulin, IgA, IgG, and IgM in serum and by reducing pathogen carriage (House, Bishop, Parry, Dougan, & Wain, 2001; House, Wain, et al., 2001; Mastroeni, Chabalgoity, Dunstan, Maskell, & Dougan, 2001). Along with these intervention strategies, good animal management or good agriculture practices are equally crucial to the production of healthy animals and agricultural products to ensure food safety.

1.3. Antibiotics and bacterial resistance

Antibiotics have been widely used in animal agriculture to control disease and to increase animal growth rate or efficiency. Although antibiotics are used to target specific bacteria, the specificity can sometimes be too narrow to be highly effective. Therefore, in several occasions, broad-spectrum antibiotics are often included in animal rations. Such treatments can disrupt the intestinal microbial ecosystem and can lead to the establishment of opportunistic pathogens. This could also impose deleterious effects on animal

health, performance, and food safety. In addition, the use of antibiotics in human and veterinary medicine to treat infectious diseases has led to the rise and spread of antibiotic resistance (Callaway, Anderson, et al., 2003; Callaway, Edrington, et al., 2003). Increased antibiotic-resistant strains can pose a significant public health hazard yielding increased frequency of treatment failures, severity of infection, prolonged duration of sickness, increase in systemic infections, and increased hospitalizations and mortality (Newell et al., 2010). Antibiotic use in plants, animals, and humans for health-promoting purposes can lead to emergence and dissemination of resistant bacteria and resistance genes. Since antibiotic resistance can spread horizontally, the use of antibiotics in one ecological compartment can have a consequence on the resistance status in another (Kruse & Sorum, 1994; Newell et al., 2010).

Food of plant or animal origin can be a source of both antibiotic-resistant bacteria and resistance genes. The presence of antibiotic-resistant bacteria in food presents a direct hazard to food handlers and consumers equally. Additionally, resistant traits can be transferred from bacteria of food origin to human pathogens directly or via a commensal resulting in an indirect hazard (Newell et al., 2010). In addition, antimicrobial resistance can also arise due to continued exposure of bacteria to antimicrobial residues in food. Furthermore, the different routes through which bacteria acquire resistance are complex. There is an increasing evidence of incidence of antibiotic-resistant bacteria in food, highlighting the importance of antibiotic-resistant foodborne pathogens and their infections. Several antibiotic-resistant strains of *Salmonella* (Threlfall, 2000), *Campylobacter* (FAO/WHO/OIE, 2003), *Shigella*, *Vibrio*, and *S. aureus* (de Boer et al., 2009), *E. coli* (Walsh et al., 2008), and *Enterococci* (FAO/WHO/OIE, 2003) have been reported for foods of plant and animal origin (Newell et al., 2010). Several of these strains have also been reported to be multidrug resistant. Infections caused by such strains are an important health problem. An important step in curbing antimicrobial resistance is the enforcement of prudent use of antimicrobial agents in all sectors of animal and food production (FAO/WHO/OIE, 2007) and to employ alternative strategies to control food pathogens.

1.4. Role of probiotics and their potential contribution to gut health and disease prevention

At present, the interrelationship between diet and health is well established. The traditional role of diet is to provide the nutrients essential for metabolism. However, over the last few decades, this idea has evolved.

It is now established that besides meeting metabolic needs, the diet also helps promote health and the state of well-being of the individual. This change in concept of the dietary role of foods has led to the development of a new class of dietary products called functional foods. A food can be considered functional if it beneficially affects target function in the body besides its nutritional value (Figueroa-Gonzalez, Quijano, Ramirez, & Cruz-Guerrero, 2011). Probiotics fall into the category of functional foods (Nagpal et al., 2012). These are nonpathogenic microorganisms that confer a health benefit on the host and prevent some diseases when administered in adequate amounts (Fric, 2007). The widely used probiotics include lactobacilli, bifidobacter, bacilli, yeast, and some nonpathogenic species within the genera of *Escherichia* (i.e., *E. coli* Nissle 1917), *Enterococcus*, and *Bacillus*. However, the most common probiotics belong to the genera *Lactobacillus* and *Bifidobacterium*. Probiotics in general exert beneficial effects in three ways: (i) Provide end products of anaerobic fermentation of carbohydrates such as organic acids that can be utilized by the host. These end products once absorbed into the blood stream are able to influence human mood, energy level, and even cognitive abilities. (ii) Effectively compete with pathogen colonization to exclude them from causing disease, and (iii) stimulate host immune system by producing specific polysaccharides.

These health benefits are generally strain specific (not species- or genera specific); thus in most cases, a cocktail of probiotics is used to gain the most benefits. Probiotics cocktail can also ensure health benefits, in the event, if one strain fails. To control foodborne pathogens, probiotics are either used in competitive exclusion or as defined cultures. Competitive exclusion involves the extrinsic administration of probiotics to food animals for intestinal colonization. Probiotic bacteria also affect the composition and function of intestinal microbial population (O'Toole & Cooney, 2008). The predominant presence of the probiotic in the gut prevents the pathogens access to the ecological niche, interfering with the attachment of pathogens to the gut and subverting the eventual infection process (Gaggia et al., 2010). Besides physical displacement of pathogens, several probiotics also produce bacteriocins, which are antimicrobial peptides that inactivate pathogens. Additionally, probiotics stimulate the immune system and help in mounting protective response against pathogen interaction with host cells (Gill & Prasad, 2008). Probiotic-induced enhanced butyrate production can also increase resistance against diseases through activation of antimicrobial host defense peptide (Floch, 2010; Sunkara et al., 2011).

Although the use of probiotics is promising, there are several challenges for the industrial production of probiotic-based functional foods: (i) improvement in production techniques for large-scale manufacturing; (ii) cost-effective production strategy; (iii) enhancement of probiotic viability during storage, manufacturing, and transit in the gastrointestinal tract; and (iv) development of efficient delivery system. Strategies to overcome these challenges must be multidisciplinary and will help make the process cost-effective and beneficial to both the producers and consumers.

1.5. Safety assessments of probiotics for therapy

Bacteria are deliberately added to food either to enhance product flavor (starter cultures) or to enhance health benefits (functional additives). Probiotic bacteria are used for the prevention or treatment of various diseases; thus, the probiotic organisms must be nonpathogenic to the host prior to its consideration for use. Commonly used probiotic bacteria include members of the genera *Lactococcus*, *Lactobacillus*, and *Bifidobacterium*. Lactic acid bacteria are generally considered safe; however, some species of *Lactobacillus* and *Bacillus* have been associated with opportunistic infections in patients with underlying conditions resulting in endocarditis, bacteremia, and liver abscess (Boyle, Robins-Browne, & Tang, 2006). Presumably, the pathogenic property is associated with a specific strain rather than with the species in general. Thus, the strain-specific characterization is essential to prove the absence of pathogenicity.

Prior to the approval of a probiotic for human use, it is essential that the bacteria be screened for potential pathogenicity and virulence traits (Sanders et al., 2010). Providing evidence for the absence of virulence properties is relatively straightforward in elucidating the pathogenic potential. Besides phenotypic characterization, it is also essential to genetically screen potential candidates for use as probiotics. Another critical consideration is the scope for antimicrobial resistance. In addition to being sensitive to antibiotics, it is also essential that the probiotic bacteria do not carry any transferrable antibiotic resistance genes, which can serve as genetic reservoirs for other potentially pathogenic bacteria. Besides acquisition of antibiotic-resistant genes, there is also the risk for uptake of virulence genes from pathogens that coinhabit the intestinal tract at the same time. However, there is no evidence in the literature of such event taking place in the gut. This could partly be due to the transient colonization of the gut by probiotics. Scientific Committee on Animal Nutrition (SCAN) has established a guideline (a decision tree, Fig. 5.1) for the approval of a probiotic strain based on antibiotic resistance (SCAN, 2002). Similar screening strategies are also employed for

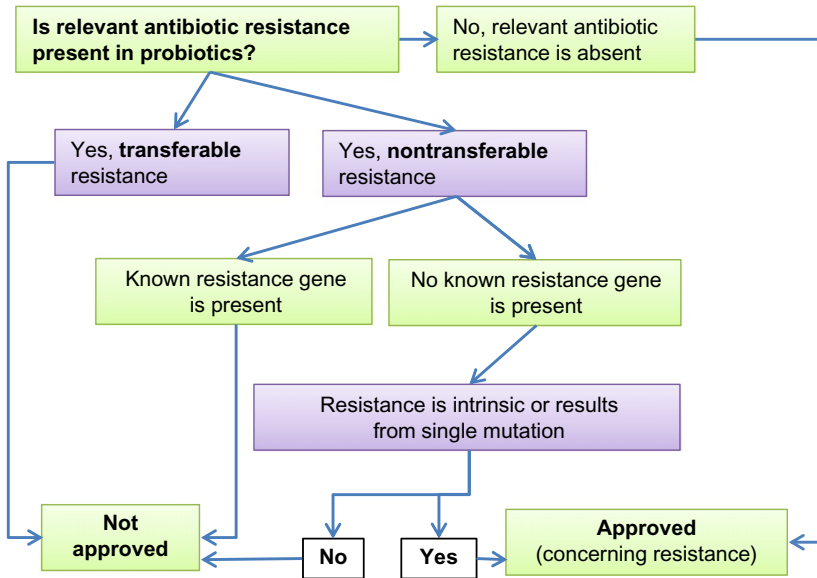


Figure 5.1 Decision network for approval of a probiotic additive based on resistance to antibiotics (SCAN, 2002). Redrawn from SCAN (2002).

the approval of a probiotic strain for use as a feed additive. Considering all the factors that are essential in the assessment of safety of probiotics, it is paramount that the general conclusion that “probiotics are safe” cannot be broadly made. Prior to the use of a probiotic or probiotic cocktail in foods or dietary supplement, they need to be determined to be safe for the general population. When intended for use as drugs, the safety assessment must balance risk with benefit (Sanders et al., 2010). The FAO has outlined a guideline for approval of a probiotic strain for use in food (Fig. 5.2).



2. PROBIOTICS

The term probiotic means “for life” and is associated with bacteria that exert beneficial effects on humans and animals. This was first observed by Eli Metchnikoff in 1907 who suggested that the dependence of intestinal microbes on food makes it possible for them to develop measures to modify the gut flora and to replace the harmful microbes with useful microbes. The initial works of E. Metchnikoff and H. Tissier in the early twentieth century set the stage for the elucidation of the beneficial effects of probiotics and their

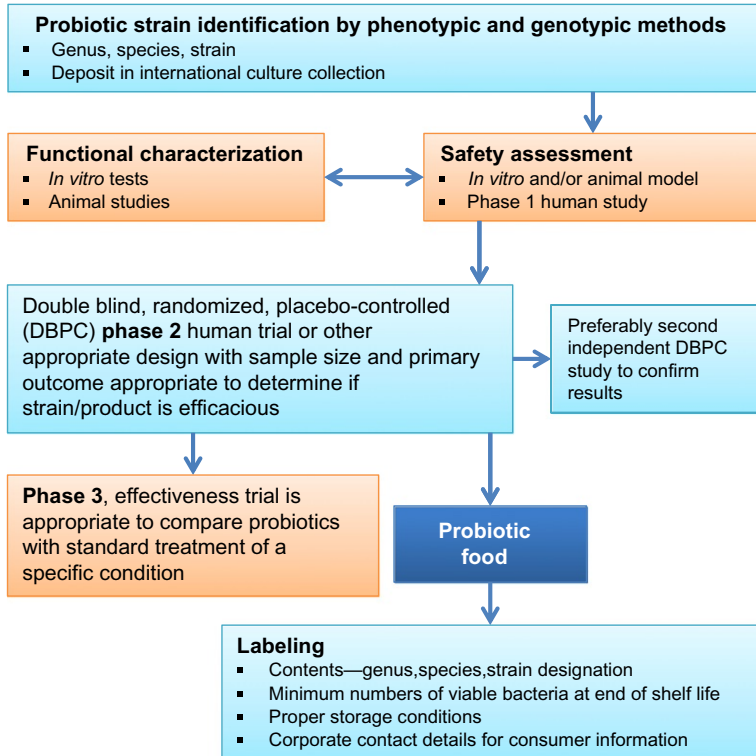


Figure 5.2 Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) guidelines for evaluation of probiotics for food use. (<ftp://ftp.fao.org/es/food/wgreport2.pdf>).

multitude of applications in human health as summarized in a recent review article (Bron, van Baarlen, & Kleerebezem, 2012). The increasing body of scientific evidence that demonstrates the beneficial effects of probiotics on health and disease prevention and treatment has made probiotics increasingly important as part of human nutrition and has led to a surge in the demand for probiotics in clinical applications (Deshpande, Rao, & Patole, 2011; Ng, Hart, Kamm, Stagg, & Knight, 2009; Vanderpool, Yan, & Polk, 2008) and as functional foods (Ly, Litonjua, Gold, & Celedon, 2011; Nagpal et al., 2012).

2.1. Definition and classifications

Probiotics have been defined based on their intent of use. Fuller (1989) defined probiotics as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance.” This definition

highlighted the microbial nature of probiotics. Similarly, Huis in't Veld, Havenaar, and Marteau (1994) defined probiotics as “a viable mono or mixed culture of bacteria which, when applied to animal or man, beneficially affects the host by improving the properties of the indigenous flora.” A more recent definition accepted by the FAO/WHO (2002) defines probiotics as “live microorganisms which, when administered in adequate amounts confer a health benefit on the host.” These definitions tend to reiterate the basic definition that probiotics are live microorganisms that in adequate dose can be beneficial to humans.

Probiotics can be classified based on their ability to colonize the intestine as resident or transient. Resident strains are those that are common inhabitants of the human digestive tract, and probiotic supplements containing these strains are able to re-establish in the intestinal tract. Resident strains may have least antagonistic effect on other beneficial resident strains in the intestinal tract. Transient strains pass through the system and do not re-establish themselves. Certain transient strains may not be effective when used as monocultures and hence in most cases are combined with other resident strains to enhance their efficacy. Taxonomically, probiotics must be identified by their genus, species, and strain as is done with other bacteria. The commonly used probiotics include the members of the genus *Lactobacillus*, *Bifidobacteria*, *Streptococcus*, *Enterococcus*, *Leuconostoc*, and yeast (*Saccharomyces*). Among these, the resident strains include *Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Bifidobacterium bifidum*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium animalis*, *Streptococcus faecalis*, and *Streptococcus faecium*. Transient strains include *Lactobacillus casei*, *Lactobacillus rhamnosus* GG, *Lactobacillus bulgaricus*, *Lactobacillus yoghurtii*, *Lactobacillus brevis*, *Lactobacillus kefir*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Streptococcus lactis*, and *Streptococcus thermophilus*.

2.2. General health benefits of probiotics

Bacteria should possess certain characteristics to be identified as a probiotic. Table 5.3 lists the criteria that are essential for a bacterium to be classified as a probiotic. To be able to produce desired beneficial effects, it has been established that a dose of 5 billion colony forming unit/day has been recommended for at least 5 days (Gronlund, Lehtonen, Eerola, & Kero, 1999; Williams, 2010). Probiotics either as mono or mixed cultures mainly consisting of *Lactobacilli* have been used for human consumption in a variety of foods such as fermented milks (yogurt), chesses, fruit juices, chocolates, wine, and sausages. Mixed cultures are highly desirable

Table 5.3 Criteria of an ideal probiotic

1. Accurate taxonomic identification
 2. Normal inhabitant of the targeted species
 3. Generally recognized as safe
 4. Resistant to bile, hydrochloric acid, and pancreatic juice
 5. Ability to survive in both acidic conditions of the stomach and the alkaline conditions of the intestine
 6. Ability to persist in the gut even if it does not colonize
 7. Adhesion to epithelium to prevent physical removal
 8. Immunostimulatory action
 9. Nonpathogenic
 10. Maintain high cell viability and metabolic activity at the target site
 11. Stability of desired characteristics during processing, storage, and delivery
 12. Genetic stability
-

because they may have synergistic effects, and moreover, if one fails, others still can exert beneficial effects.

It is well established that probiotics have several beneficial attributes (Dicks & Botes, 2010; Nagpal et al., 2012; Williams, 2010). Those include lactose metabolism and food digestion, production of antimicrobial peptides and control of enteric infections, antimycotic effects, anticarcinogenic properties, immunologic enhancement, enhancement of short-chain fatty acid (SCFA) production, antiatherogenic and cholesterol-lowering attributes, regulatory role in allergy (Thomas et al., 2011), protection against vaginal or urinary tract infections, increased nutritional value, maintenance of epithelial integrity and barrier, stimulation of repair mechanism in cells, and maintenance and reestablishment of a well-balanced indigenous intestinal and respiratory microbial communities.

2.3. Mechanism of probiotic action

Several mechanisms have been proposed regarding action of probiotics. Some of the major attributes are discussed below (Table 5.4).

2.3.1 Enhancing barrier function

The intestinal barrier function is an important defensive mechanism of the intestinal epithelium to maintain its protective effects to protect against invading pathogens and other harmful agents (Ohland & MacNaughton, 2010). The barrier function is maintained by several mechanisms that include mucus secretion, chloride and water secretion, and maintenance of cell-cell tight junctions (Thomas & Ockhuizen, 2012). Disruption in the barrier

Table 5.4 Health benefits of probiotic bacteria and their proposed mechanisms

Health benefits	Proposed mechanism
Resistance to enteric pathogens	<ul style="list-style-type: none"> • Antagonism • Increasing antibody production • Colonization resistance • Limiting access of enteric pathogens (pH, bacteriocins, antimicrobial peptides, lactic acid production)
Aid in lactose metabolism	<ul style="list-style-type: none"> • Bacterial lactase acts on lactose in the small intestine
Small bowel bacterial overgrowth	<ul style="list-style-type: none"> • Decrease toxic metabolite production • Normalize small bowel flora • Antibacterial characteristics
Immune system modulation	<ul style="list-style-type: none"> • Strengthening of nonspecific and antigen-specific defense • Regulate/influence Th1/Th2 cells activation • Production of anti-inflammatory cytokines
Anticolon cancer effect	<ul style="list-style-type: none"> • Antimutagenic and anticarcinogenic activity • Detoxification of carcinogenic metabolites • Stimulation of immune function
Decreased detoxification/excretion of toxic microbial metabolites	<ul style="list-style-type: none"> • Increased bifidobacterial cell counts and shift from a preferable protein- to carbohydrate-metabolizing microbial community
Antiallergic activity (eczema or atopic dermatitis, asthma)	<ul style="list-style-type: none"> • Prevention of antigen translocation into blood stream • Prevent excessive immunologic responses to increased amount of antigen
Blood lipids, heart disease	<ul style="list-style-type: none"> • Assimilation of cholesterol by bacterial cell • Alteration in the activity of bile salt hydrolase (BSH)
Urogenital infections	<ul style="list-style-type: none"> • Adhesion to urinary and vaginal tract cells • Competitive exclusion
Necrotizing enterocolitis	<ul style="list-style-type: none"> • Decrease in TLRs and signaling molecules and increase in negative regulations • Reduction in IL-8 response
Rotavirus gastroenteritis	<ul style="list-style-type: none"> • Increased IgA response to the virus
Inflammatory bowel disease	<ul style="list-style-type: none"> • Enhancement of mucosal barrier function
Crohn's disease	<ul style="list-style-type: none"> • Reduction in proinflammatory cytokines production

Adapted from Nagpal et al. (2012)

function can lead to various conditions such as inflammatory bowel disease (IBD), coeliac disease, enteric infections, and other autoimmune diseases (Ng et al., 2009). Several probiotics have been shown to protect the epithelial barrier and prevent mucosal damage triggered by food antigens, enteric pathogens, drugs, and proinflammatory cytokines (O'Hara & Shanahan, 2007). These protective effects are mediated through several mechanisms either directly or indirectly through alteration of gut microflora populations.

The first barrier that the intestinal bacteria and pathogens meet is the mucus. The entire length of the intestinal tract is lined by goblet cells. The percent of goblet cells increase from duodenum (4%) to descending colon (~16%) relative to the epithelial cells (Goto & Kiyono, 2012). Intestinal microflora also regulates the goblet cell populations in the gut. Goblet cells secrete mucin that are resistant to proteolysis and form a protective gel layer over the epithelial surface. Intestinal bacteria and pathogens have to penetrate the mucus layer to reach the epithelial cells during infection. Several microorganisms have developed diverse methods to degrade mucus either to aid in invasion or for uptake of mucus-derived nutrients (Aristoteli & Willcox, 2003; Ohland & MacNaughton, 2010). Additionally, several studies have also reported that the mucus layer is significantly thinner in areas of inflammation thus compromising the barrier and allowing for increased bacterial adherence and infiltration (Swidsinski et al., 2007). Probiotics enhance the barrier by promoting mucus secretion. Several *Lactobacillus* species have increased mucin expression in *in vitro* cell culture models and have blocked pathogenic *E. coli* adherence and invasion (Mack, Ahrne, Hyde, Wei, & Hollingsworth, 2003). Similarly, *in vivo* experiments in rats fed with probiotic cocktail VSL#3 for 7 days demonstrated a significant increase in mucin secretion (Caballero-Franco, Keller, De Simone, & Chadee, 2007). Thus, enhanced mucus production by probiotics *in vitro* and *in vivo* could be used as a protective strategy to augment the intestinal barrier function.

Once the bacteria come across the mucus layer, they find binding sites on the epithelium for colonization/attachment. Pathogenic bacteria, however, proceed to penetrate or damage the epithelium to cause disease. The enterocytes express pattern recognition receptors such as Toll-like receptors (TLRs) that sense the presence of conserved bacterial motifs and initiate cascade of proinflammatory signals (Franchi, Wamer, Viani, & Nunez, 2009). These receptors are present intracellularly and basolaterally on enterocytes. Therefore, only after a pathogen breaches the barrier, they can come in contact with the receptors, which can differentiate between commensal and

pathogenic bacteria in the gut (Franchi et al., 2012; Franklin & Latz, 2012). Enterocyte cell–cell adhesion is an essential component of the intestinal barrier. Several components make up the cell–cell junctional complexes such as tight junctions, adherens junctions, gap junctions, and desmosomes (Turner, 2009). These intercellular junctional complexes help maintain the epithelial barrier permeability and its integrity (Groschwitz & Hogan, 2009; Marchiando, Graham, & Turner, 2010; Ohland & MacNaughton, 2010). Regulation of tight junctions and the associated epithelial permeability is essential to maintain the epithelial barrier function. However, pathogens have evolved different mechanisms to cross the epithelial barrier demonstrating the critical role the barrier plays in maintenance of homeostasis and prevention of inflammation (Goto & Kiyono, 2012). Chronic inflammation has been shown to be associated with altered tight junction barrier function that can promote pathogen access to the basolateral side of the epithelial barrier.

Several studies have demonstrated that pretreatment with probiotic bacteria can inhibit the loss of permeability associated with tight junction alteration caused by stress, infection, or proinflammatory cytokines (Ait-Belgnaoui et al., 2006; Dahan et al., 2003; Ewaschuk et al., 2008; Sherman et al., 2005). It has been shown that probiotics can directly alter epithelial barrier function by altering the structure and function of tight junctions. Resta-Lenert and Barrett (2003, 2006) found that pretreatment with *S. thermophilus* and *L. acidophilus* independently decreased the permeability of cell monolayers formed by intestinal cells of HT-29 and Caco-2. This study also demonstrated that probiotics altered the expression of several proteins that are structural components of tight junctions thereby decreasing permeability. Another study by Yan et al. (2007) demonstrated that certain proteins produced by probiotic bacteria can interact with mammalian cell signaling proteins and lead to alteration in tight junction function and permeability. Two such proteins produced by *L. rhamnosus* (p40 and p75) inhibited apoptosis in enterocytes and promoted survival. In addition, these proteins also altered structural components of the cell junctional complexes and enhanced barrier function (Table 5.5).

2.3.2 Immunomodulation

The intestine is the first site for foreign antigen encounter. Therefore, the intestine has developed a tightly regulated mechanism to protect against pathogen invasion. The intestinal immune system is made up of several

Table 5.5 Summary of probiotics effects on epithelial barrier function *in vitro* and *in vivo*

Barrier function and probiotic	Effect	Model	Reference
<i>Mucous layer</i>			
Lactobacillus	↑ MUC2 and/or 3 expression	Caco-2, HT29	Kim et al. (2008), Mattar et al. (2002)
VSL#3	↑ MUC2, 3, and 5AC expression (no effect on MUC1)	HT29	Otte and Podolsky (2004)
VSL#3	↑ MUC1, 2, and 3 expression and secretion	Rat	Caballero-Franco et al. (2007)
<i>Tight junctions</i>			
<i>S. thermophilus</i> , <i>L. acidophilus</i>	↑ TER, ↓ permeability; activation of occludins, ZO-1, ERK 1/2	HT29, Caco-2	Resta-Lenert and Barrett (2003)
<i>B. infantis</i>	↑ TER, ↓ permeability; ↑ ZO-1, occludin, ↓ claudin-2 expression; prevent IFN- γ and TNF- α effects	T84	Ewaschuk et al. (2008)
<i>Escherichia coli</i> Nissle	↑ ZO-1 expression; prevent DSS-induced decrease in permeability and illness	DSS-treated mouse	Ukena et al. (2007)
<i>Saccharomyces cerevisiae</i> (Boulardii)	Prevent EHEC-induced apoptosis	T84	Dalmaso et al. (2006)
<i>L. rhamnosus</i> p40, p75	Inhibit cytokine-induced apoptosis	YAMC, HT29, mouse colon explant	Yan et al. (2007), Yan and Polk (2011)
<i>L. rhamnosus</i> p40, p75	Inhibit H ₂ O ₂ -induced ↓ TER and ↑ permeability	Caco-2, HT29, T84	Seth, Yan, Polk, and Rao (2008)

↑, increased; ↓, decreased; EcN, *Escherichia coli* Nissle; EHEC, enterhemorrhagic *E. coli*; TER, transepithelial resistance; ZO, zonula occludens; TJ, tight junction; DSS, dextran sodium sulfate. VSL#3 contains four *Lactobacillus* spp. (*L. acidophilus*, *L. casei*, *L. plantatarum*, *L. delbrueckii*), three *Bifidobacterium* spp. (*B. infantis*, *B. longum*, *B. breve*), and one *Streptococcus salivarius* subsp. *thermophilus*.

Adapted from Ohland and MacNaughton (2010).

lymphoid organs collectively called as the gut-associated lymphoid tissue. This is the largest collection of lymphoid tissues in the body and consists of the mesenteric lymph nodes, Peyer's patches, isolated lymphoid follicles, lymphocytes, and dendritic cells (Forchielli & Walker, 2005; Hakansson & Molin, 2011; Newberry & Lorenz, 2005). Microbial colonization of the gut affects the composition of immune cell populations. Several studies have demonstrated that bacterial colonization of the gut led to an increase in the number of intraepithelial lymphocytes, germinal centers with antibody-producing cells, and serum antibody concentrations (Hakansson & Molin, 2011). This demonstrates the complex relationship that exists between the intestinal immune system and the gut microbiota (Bron et al., 2012).

Several *in vivo* and *in vitro* studies have demonstrated the immunostimulatory effects that probiotics have on the intestinal immune system (Gill & Prasad, 2008). Probiotics and probiotics-derived products are detected by the specialized membranous cells (M cells). Antigens taken up by the M cells are processed by the antigen-processing cells (APCs) and presented to naïve T cells. The type of cytokine secreted, phenotype, and activation of APCs determine the lineage of the T cell that is produced, namely T helper 1 (Th1), T helper 2 (Th2), or the T regulatory (Treg) cells. Activation of Th1 cells leads to production of IFN- γ , TNF- α , and IL-2 which leads to the development of cell-mediated and cytotoxic immunity. Th2 cells activated by APCs mainly secrete IL-4, IL-5, and IL-13 which promote antibody production. Treg cells secrete IL-10 and TGF- β , which downregulate activities of both Th1 and Th2 cells and help maintain homeostasis in the intestine (Gill & Prasad, 2008).

Probiotics have been demonstrated to modulate the innate and acquired immune responses. The innate immune system forms the first line of defense against pathogens. The major components of the innate immune system include epithelial cells, phagocytic cells (monocytes, macrophages, neutrophils), and natural killer cells. Several clinical trials have demonstrated that probiotics enhance the phagocytic activity of peripheral blood leukocytes (Gill, 2003). Healthy subjects administered with *L. johnsonii*, *L. rhamnosus*, or *B. lactis* demonstrated an enhanced phagocytic capacity of peripheral blood leukocytes. The increase in phagocytic ability was also found to be dose dependent and lasted for several weeks after cessation of probiotic intake (Gill & Rutherford, 2001b). Probiotics also can activate neutrophils through increased expression of phagocytosis receptors (Pelto, Isolauri, Lilius, Nuutila, & Salminen, 1998) and an increased oxidative burst or microbicidal

capacity of leukocytes (Parra, de Morentin, Cobo, Mateos, & Martinez, 2004). Similarly, consumption of probiotics by healthy subjects led to an increase in the number and activity of NK cells and increased phagocytic action in animals fed with probiotic supplements (Cross, 2002). Probiotic-mediated gut health is attributed to the stimulation of epithelial innate immunity. Administration of probiotic mixture VSL#3 exhibited anti-inflammatory effect by stimulation of epithelial-derived TNF- α production and activation of NF- κ B (Pagnini et al., 2010).

Besides modulating the innate immune response, probiotics have also been shown to augment the acquired immune responses through induction of cell-mediated immunity. Intake of probiotics has led to an increase in antibody responses to natural infections and immunizations. A randomized trial in children with rotavirus administered with *L. rhamnosus* GG demonstrated an increase in specific mucosal and serum antibody responses (Kaila et al., 1992). Similarly, administration of probiotics following immunization with *Salmonella* vaccine in subjects led to a significantly higher specific serum IgA and IgA-mediated cell responses (Linkamster, Rochat, Saudan, Mignot, & Aeschlimann, 1994). These observations indicate that probiotic strains exhibit adjuvant properties increasing the efficacy of antibody production and immune responses to immunizations. Probiotics mediate these effects through increased transport of antigenic materials across the gut mucosa and upregulation of antigen-presenting molecules and costimulatory molecules in immune cells (Gill & Prasad, 2008; Hakansson & Molin, 2011).

An important component of the immune response mediators are cytokines. They are the largest and the most pleiotropic group of mediators. They are responsible for initiation, maintenance, and resolution of innate and acquired immune responses. Several studies have demonstrated that ability of specific probiotic strains to enhance cytokine production and influence both innate and acquired immune responses. Probiotic administration has been reported to enhance levels of IFN- γ , IFN- α , and IL-12 in healthy subjects (Arunachalam, Gill, & Chandra, 2000). Long-term consumption of probiotic containing yogurt has been shown to increase production of IL-1 β , IL-6, IL-10, IFN- γ , IL-1, TNF- α , IL-10, IL-12, IL-18, and TGF- β by mononuclear cells and dendritic cells (Cross, 2002; Gill & Guarner, 2004; Niers et al., 2005). Several studies have provided direct evidence that the administration of specific probiotic strains can help in the prevention and treatment of gastrointestinal infections. Studies conducted in animals have also demonstrated the ability of probiotics to enhance serum and mucosal antibodies, phagocytic cell function and

NK cell activity, and resistance to infection with pathogens (Gill & Prasad, 2008; Hakansson & Molin, 2011) (Table 5.6).

2.3.3 Antimicrobial action and cytoprotective effects

Probiotics exert their antimicrobial and cytoprotective effects on host intestinal epithelium directly through the production of antimicrobial factors and indirectly through the increase in expression of host cell antimicrobial peptides, enhancement of barrier function, and immunomodulation. This section specifically discusses the antimicrobials produced by probiotics and their antagonistic effect on pathogens. The intestinal cells produce two main classes of antimicrobial peptides, namely, defensins and

Table 5.6 Immunomodulatory effects of probiotics

Immune function and probiotic	Effect	Model	Reference
<i>L. casei</i>	↑ Levels of IgA+ and IL-6-producing cells in the lamina propria	Mouse	Galdeano and Perdigon (2006)
<i>L. casei</i>	No change	Monoassociated mouse	Martins et al. (2009)
<i>B. animalis</i> , <i>E. coli</i> EMO	↑ Total sIgA	Monoassociated mouse	Martins et al. (2009)
<i>B. bifidum</i> and <i>B. infantis</i>	↑ Levels of rotavirus-specific sIgA	Mouse	Qiao et al. (2002)
<i>B. lactis</i>	↑ Levels of EHEC-specific sIgA	Mouse	Shu and Gill (2001)
<i>L. casei</i>	↑ Levels of EHEC- or Shiga toxin-specific sIgA leading to increased survival	Rabbit	Ogawa et al. (2001)
<i>L. helveticus</i>	↑ Lamina propria IgA+ B cells and sIgA	Rat	LeBlanc, Fliss, and Matar (2004)
<i>Saccharomyces cerevisiae</i> (Boulardii)	↑ Total sIgA	Conventional and monoassociated mouse	Martins et al. (2009)

↑, increased; ↓, decreased; EHEC, enterhemorrhagic *E. coli*; sIgA, secretory IgA.

Adapted from Ohland and MacNaughton (2010).

cathelicidins. Cathelicidins are constitutively produced by intestinal epithelial cells to aid in host defense against pathogens (Kelsall, 2008). The only stimulus that appears to induce cathelicidin production is butyrate that is produced by intestinal flora (Schauber et al., 2003) and also by probiotics (Floch, 2010; Sunkara et al., 2011). *Shigella* infection (dysentery) in rabbits was significantly reduced by feeding butyrate to induce cathelicidin production (Raqib et al., 2006).

Defensins produced by the intestinal cells can be classified as α -defensins and β -defensins. α -Defensins are produced by small bowel Paneth cells (HD-5 and HD-6), and β -defensins are expressed by epithelial cells throughout the intestine (kBD-1 through 4). These defensins exhibit antimicrobial activity against a wide variety of bacteria, fungi, and viruses. Defensins are constitutively produced in the intestines to keep pathogens from reaching the epithelium (Wehkamp et al., 2004). *In vitro* studies with probiotics have demonstrated that Caco-2 cells upon stimulation by *E. coli* Nissle, *E. coli* strain DSM 17252, and several Lactobacilli in the cocktail VSL#3 led to an increased expression and secretion of human β defensin-2 (hBD-2) (Schlee et al., 2008; Wehkamp et al., 2004). The underlying mechanism through which probiotics stimulated hBD-2 production was elucidated using specific inhibitors. It was observed that hBD-2 secretion was enhanced through activation of MAP kinases (Schlee et al., 2008). Besides activation of MAP kinases, it was also observed that *E. coli* Nissle flagellin also stimulated hBD-2 production (Schlee et al., 2007).

In addition to stimulating the production of host antimicrobial peptides, probiotics by themselves produce several antimicrobial compounds such as bactericidal peptides and SCFAs that can directly inactivate pathogens (Dobson et al., 2012; Hassan et al., 2012). These secreted factors can be considered an integral part of the intestinal barrier. SCFAs include acetic and lactic acid which reduce the luminal pH resulting in the growth inhibition of some pathogens including enterohemorrhagic *E. coli* (EHEC) and *Salmonella enterica* serovar Typhimurium *in vitro*. Additionally, SCFAs produced by probiotics have also been shown to decrease Shiga toxin gene expression by *E. coli* O157:H7 (Carey, Kostrzynska, Ojha, & Thompson, 2008; Fayol-Messaoudi, Berger, Coconnier-Polter, Lievin-Le Moal, & Servin, 2005). Furthermore, SCFAs can also disrupt the outer membrane of Gram-negative pathogens such as EHEC, *Pseudomonas aeruginosa*, and *S. Typhimurium* thereby inhibiting pathogen growth. Increased permeabilization of the outer membrane of pathogens also potentiates the activity of other antimicrobial

molecules by aiding in their penetration of the cell wall (Alakomi et al., 2000). Besides lactic acid, the predominant antimicrobial activity in *Lactobacilli* is through the production of antimicrobial peptides, bacteriocins, and microcins (Fayol-Messaoudi et al., 2005). Bacteriocins and microcins are peptides with bactericidal or bacteriostatic activity produced in a strain-specific manner by probiotics (Lievin-Le Moal & Servin, 2006). Bacteriocins are peptides produced by Gram-positive bacteria while microcins are produced by Gram-negative bacteria. Bacteriocins permeabilize the cytoplasmic membrane of bacteria leading to disruption of cell wall synthesis and formation of pores eventually leading to cell death. Microcins, on the other hand, target the enzymes that are involved in DNA or RNA structure and synthesis or protein synthesis enzymes (Duquesne, Petit, Peduzzi, & Rebuffat, 2007). Collectively, these antimicrobial compounds protect the intestinal barrier by rapidly eliminating pathogens from the gut.

Bacteriocin ABP-118 produced by *L. salivarius* has been shown to inhibit the growth of *Bacillus*, *Listeria*, *Staphylococcus*, and *Enterococcus* species. However, this bacteriocin did not affect the growth of most *Lactobacillus* species thereby providing a selective advantage for intestinal colonization of probiotic and commensal bacteria (Flynn et al., 2002). Similarly, Lacticin 3147 produced by *L. lactis* has been shown to *Clostridium difficile* as a potential therapy; however, this bacteriocin inhibited other resident bacteria including *Lactobacillus* and *Bifidobacteria* (Rea et al., 2007). In a separate study, Banerjee, Merkel, and Bhunia (2009) showed that the soluble factors produced by probiotic *Lactobacillus delbrueckii* subsp. *bulgaricus* were able to neutralize *C. difficile* toxin, thereby preventing cytotoxicity in a cell culture model. *L. delbrueckii* has been demonstrated to produce hydrogen peroxide that can inactivate pathogens by oxidation. *L. delbrueckii* also produces lactic acid, heat-sensitive, and heat-resistant bacteriocins. The heat-resistant bacteriocin has been observed to inhibit the growth of *S. thermophilus* (van de Guchte, Ehrlich, & Maguin, 2001). Similarly, certain *Bifidobacterium* strains produce lipophilic molecules that have been shown to inhibit the viability of *E. coli*, *Klebsiella pneumoniae*, *Yersinia pseudotuberculosis*, *S. aureus*, and *S. Typhimurium*. Additionally, this antimicrobial compound has also been demonstrated to prevent invasion of Caco-2 cells by *S. Typhimurium* and can also kill intracellular *S. Typhimurium* in a therapeutic model (Lievin et al., 2000).

Probiotics also exert their antimicrobial and cytoprotective effect by inhibiting pathogen adherence to the intestinal epithelium (Collado, Isolauri, Salminen, & Sanz, 2009; Sherman, Ossa, & Johnson-Henry,

2009). Probiotics inhibit pathogen adherence by competing for the binding sites on the epithelial cells. Pretreatment of HEp-2 and T84 cell lines with *L. rhamnosus* and *L. acidophilus* significantly reduced the binding of enteropathogenic *E. coli* (EPEC) and EHEC to the monolayers. In addition, probiotic pretreatment also reduced EHEC-induced increase in permeability and helped maintain monolayer integrity (Johnson-Henry, Donato, Shen-Tu, Gordanpour, & Sherman, 2008; Sherman et al., 2005). Probiotic bacteria or its cell surface components also inhibited *E. coli* O157:H7 adhesion to Caco-2 cells (Johnson-Henry, Hagen, Gordonpour, Tompkins, & Sherman, 2007; Medellin-Pena & Griffiths, 2009) or virulence-associated gene expression (Medellin-Pena, Wang, Johnson, Anand, & Griffiths, 2007). *Lactobacillus* strains have also been shown to compete directly with pathogens such as *Salmonella* species, for binding sites on human mucins or Caco-2 cell surfaces (Gueimonde, Jalonon, He, Hiramatsu, & Salminen, 2006). Besides physical displacement of pathogens, *E. coli* Nissle has been shown to secrete a nonbacteriocin component that may act either on the pathogen or on the host cell to inhibit adherence of several pathogens (Altenhoefer et al., 2004). Similar studies performed in a rat model suffering from chronic psychological stress that were administered with *L. rhamnosus* and *L. helveticus* demonstrated reduced commensal bacterial adherence and translocation (Zareie et al., 2006). Thus, inhibition of pathogen adherence is another mechanism through which probiotic bacteria prevent intestinal infection. Altogether, probiotic exerts its cytoprotective effect in the intestinal tract through their ability to enhance intestinal barrier function, immune modulation, toxin binding and neutralization, and inactivation and prevention of pathogen attachment (Table 5.7).



3. INTERACTION OF GUT MICROBIOTA AND PROBIOTICS

When a probiotic is administered orally, it first encounters various harsh environments in the gut. Therefore, it is essential that probiotics survive in the intestinal tract in significant numbers to produce beneficial effects. Survival of the probiotic is dependent on multiple factors such as stress response, metabolism, pH homeogenesis, cell wall maintenance, and fatty acid synthesis (Breton et al., 2002; Sanders, 2011). Gram-positive probiotic bacteria use several mechanisms to help them survive in the gut. These include proton pumps, amino acid decarboxylation, and electrogenic transport systems that aid in acid resistance, changes in the

Table 5.7 *In vivo* effect of bacteriocins against enteric pathogens

Bacteriocin	Producer strain	Animal model	Activity	Reference
Bacteriocin B602	<i>Paenibacillus polymyxa</i> NRRL B-30509	Chicken	Inhibitory to <i>Campylobacter jejuni</i>	Stern et al. (2005)
Mutacin B-Ny266	<i>Streptococcus mutans</i> Ny266	Mice	Reduced mortality due to <i>S. aureus</i>	Mota-Meira, Morency, and Lavoie (2005)
Enterocin A	<i>Enterococcus faecium</i> EK13	Japanese quails	Reduced <i>Salmonella</i> concentration and associated intestinal damage	Cigankova, Laukova, Guba, and Nemcova (2004)
E-760	<i>Enterococcus</i> sp. NRRL B-30745	Broiler chicks	Reduced colonization by <i>C. jejuni</i>	Line et al. (2008)
Bacteriocin E 50-52	<i>Enterococcus faecium</i> NRRL B-30746	Broilers	Reduced colonization by <i>C. jejuni</i> and <i>Salmonella enteritidis</i>	Svetoch et al. (2008)
Bacteriocin OR7	<i>Lactobacillus salivarius</i> NRRL B-30514	Turkeys	Reduced <i>Campylobacter</i> concentrations	Cole et al. (2006)
Bacteriocin PPB 7420	<i>Enterococcus faecium</i> CCM 7420	Rabbits	Reduced coagulase positive <i>Staphylococcus</i> in the cecum	Simonova et al. (2009)

Adapted from Bogovic-Matijasic and Rogelj (2011).

structure of their cell envelop, chaperones involved in repair of damaged proteins, and incremental expression of regulators promoting global gene responses (Cotter & Hill, 2003). In addition, several probiotics have also been shown to modulate their gene expression *in vitro* under simulated gastrointestinal tract environment or *in vivo* in mouse model (Bron, Granette, Mercenier, de Vos, & Kleerebezem, 2004; Bron, Molenaar, Vos, & Kleerebezem, 2006).

Once the probiotics survive the transit through stomach and intestine, it interacts with the major component of the gastrointestinal tract that is the resident commensal flora consisting of 500–1000 of different microbial species (Collado et al., 2009). One of the earliest investigations into the interaction between probiotics and the resident microbiota by Tannock et al. (2000) revealed that administration of *L. rhamnosus* DR20 resulted in modest fluctuations in resident *Lactobacillus* and *Bifidobacterium* numbers. Most subjects ceased shedding the probiotic strain once the administration was stopped. However, one subject continued to shed the probiotic strain for over 2 months after the test period, indicating that there are interhost variables such as bacterium–host interactions. Probiotics are commonly applied in companion and farm animals as growth enhancers (Patterson & Burkholder, 2003; Swanson & Fahey, 2006). It has been demonstrated that administration of a cocktail of *Lactobacilli*, *Bifidobacteria*, *Enterococci*, and *Pediococi* improved weight gain in broiler chickens associated with an increase in *Bifidobacterium*, *Lactobacilli*, and Gram-positive cocci populations (Mountzouris et al., 2007). A similar study in piglets administered with *Enterococcus faecium* strain reduced *Enterococcus faecalis* population in the intestine of weaning pigs, but the total numbers of *E. faecium* remained unchanged, suggesting that the *E. faecium* strain introduced had displaced part of the same species (Vahjen, Taras, & Simon, 2007). Another study performed in mice demonstrated that administration of *L. casei* and *L. plantarum* affected the overall diversity of the murine intestinal *Lactobacilli* but not the overall bacterial community structure (Fuentes et al., 2008). Furthermore, an increase in the population of *Lactobacilli* related to the acidophilus complex was observed.

Use of animal models provides us with an insight into the interaction between probiotics and the resident flora; however, studies in humans are essential if that species is the desired host. Few studies have been done on humans. Alterations in gut microflora have been reported in humans with irritable bowel syndrome (IBS) (Kassinen et al., 2007; Satokari et al., 2003), and administration of multispecies probiotic supplement was able to alleviate IBS with a stabilization of the gut microbiota over time (Kajander et al., 2007). Besides treating inflammatory conditions, probiotics have also been efficacious in the treatment of infectious diarrhea (Benchimol & Mack, 2004; Guandalini, 2008a, 2008b). There are several ways in which probiotics can alter the microbiota, which include competition for nutrients, changes in microenvironment,

production of growth substrates, direct antagonism, competitive exclusion, barrier function, immune stimulation, and reduction of inflammation (O'Toole & Cooney, 2008). Studies using transcriptional microarrays have shown that introducing a probiotic into the mouse gut changed the metabolic pathway of the endogenous microbiota (Sonnenburg, Chen, & Gordon, 2006). Gnotobiotic mice colonized by *Bacteroides thetaiotaomicron* were challenged with *B. animalis* or *L. casei*, resulting in shifts in gene expression pattern of *B. thetaiotaomicron*. Many of the altered genes were found to be involved in carbohydrate metabolism (Sonnenburg et al., 2006). This suggests that probiotics alter resident microbiota population or gene expression through competition for substrate availability and by altering the dynamics of carbohydrate utilization (Keeney & Finlay, 2011).

Probiotics can also alter the microenvironment of microbiota through a diverse range of metabolic pathway outcomes. Colonization of germ-free mice by microbiota from human baby exposed to *Lactobacilli* strains resulted in microbiome modification measured by selected culture regimes (Claus et al., 2008). This was also associated with changes in fecal levels of choline, acetate, ethanol, unconjugated bile acids, and cecal concentrations of SCFAs. Besides changing the microenvironment, several probiotic strains are also known to produce vitamins and growth factors. The enhanced availability of such growth factors can also modulate the diversity of intestinal microbiota. Probiotic bacteria also impact the resident microbiota by direct antagonism. Natural competition between probiotics and opportunistic pathogens could also be mediated through the production of bacteriocins that are exploited to modulate microbiota. The indirect mechanisms through which probiotics modulate inherent microbiota include enhancement of intestinal barrier function that can alter release rates of host-derived micronutrients and suppression of proinflammatory cytokines resulting in a reduction of gut inflammation (Zyrek et al., 2007). Reduction in inflammation can alter the gut environment sufficiently to impact on the microbiota. Additionally, administration of probiotics can also bolster the innate and acquired immune responses leading to subtle changes in the overall composition of the gut microbiota (Gill & Rutherford, 2001a, 2001c). There is adequate information in literature to indicate that administration of probiotics in high dosage impacts the diversity of gut microflora (Preidis et al., 2012). However, detailed studies are required to understand the interplay of diet, microbiota, and host factors in determining the outcomes to allow its manipulation.

4. WILD-TYPE AND BIOENGINEERED PROBIOTICS TO CONTROL FOODBORNE ENTERIC PATHOGENS

Foodborne pathogens are a major concern worldwide due to increased mortality and morbidity (Flint et al., 2005). Thus, various affordable intervention strategies including improved food-processing methods along with probiotic-based natural and functional food systems must be developed to protect people against foodborne infections. Probiotics are live nonpathogenic microorganisms that are administered to maintain and to improve intestinal microbial balance and also to protect the consumers from untoward infection from pathogens. Among the various etiologic agents, bacterial, viral, and mycotoxins are of major concerns. Several studies have demonstrated the efficacy of either wild-type or recombinant probiotics against foodborne pathogens thereby help improving animal health and preventing foodborne infections (Bhunia, 2012; Dobson et al., 2012; Paton, Morona, & Paton, 2006; Salminen et al., 2010). This section discusses the various probiotic-based intervention strategies in controlling foodborne pathogens. Mechanism of probiotic-mediated antimicrobial action in gastrointestinal tract is illustrated in Fig. 5.3.

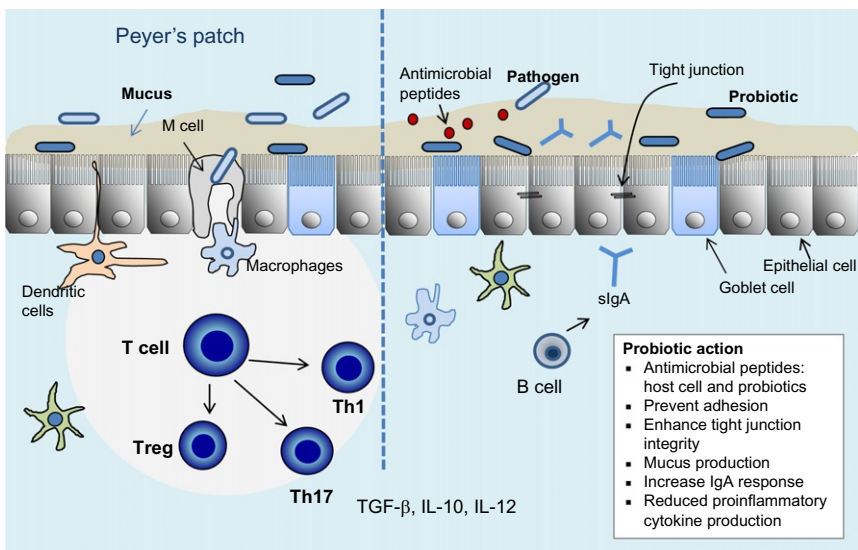


Figure 5.3 Mechanism of probiotic action against foodborne pathogens in the gastrointestinal tract depicting immunological and cellular responses.

4.1. Probiotics to control bacterial pathogens

4.1.1 *E. coli* O157:H7

EHEC is commonly implicated in foodborne illness. Control of *E. coli* O157:H7, a predominant EHEC, is particularly important because of its low infectious dose, acid tolerance, and is harbored in healthy cattle (Ferens & Hovde, 2011; Soon et al., 2011). Among the many intervention strategies investigated, probiotics are found to be effective. Lema, Williams, and Rao (2001) demonstrated the efficacy of several probiotics including *L. acidophilus*, *L. casei*, *L. fermentum*, *L. plantarum*, and *E. faecium* in reducing *E. coli* O157:H7 shedding by sheep. The microbial supplements were fed with freeze-dried fermentation products of the probiotics for a period of 7 weeks. The animal group fed with the probiotic supplement showed a significantly lower numbers of *E. coli* O157:H7 shedding in the feces and an increase in average daily weight gain and feed conversion. In addition to reducing fecal shedding, administration of *Bifidobacteria* in mice showed a decrease in Shiga toxin production. Mice that were fed with *Bifidobacterium breve* had a significant reduction in body weight loss and mortality compared to the control group demonstrating that *B. breve* is capable of protecting mice from *E. coli* O157:H7 infection (Asahara et al., 2004). A study conducted by Stephens, Loneragan, Karunasena, and Brashears (2007) also demonstrated that direct-fed probiotics consisting of *L. acidophilus* at different doses led to a dose-dependent decrease in fecal shedding and presence on the hide. In addition to the use of *Lactobacillus*, studies using nonpathogenic *E. coli* such as *E. coli* 1307 and Nissle strains also inhibited bacterial growth and Shiga toxin production by STEC (Reissbrodt et al., 2009).

Studies using *in vitro* cell culture models have also been used to elucidate the efficacy of probiotics in controlling *E. coli* O157:H7 (Sherman et al., 2005). Intestinal cell monolayers (HEp-2 and T84) were exposed to *L. acidophilus* R0052 and *L. rhamnosus* R0011 followed by infection with EHEC or EPEC (*E. coli* O157:H7 or *E. coli* O127:H6), respectively. Following infection, the adherence and cytotoxicity induced by EHEC and EPEC were examined. Exposure to the probiotic strains significantly reduced pathogen attachment to the monolayers. In addition, the probiotics also protected the monolayers from pathogen-induced loss of transepithelial resistance and tight junction integrity (Sherman et al., 2009). Besides reducing the adherence and cell injury, *L. acidophilus* cell-free spent media resulted in downregulation of several virulence genes involved in the colonization of EHEC (Medellin-Pena et al., 2007). Similar studies conducted using *E. coli*

O157:H7-infected BALB/c mice demonstrated that preexposure to *L. paracasei* resulted in an upregulation of dendritic cells and helper T cell, antibody production, and downregulation of proinflammatory cytokines yielding enhanced protection of the intestinal integrity (Tsai, Cheng, & Pan, 2010).

In addition to using wild-type probiotics, Paton et al. (2005) generated a recombinant nonpathogenic *E. coli* carrying chimeric lipopolysaccharide capable of binding enterotoxin produced by enterotoxigenic *E. coli* (ETEC). The recombinant probiotic was able to neutralize 93% of enterotoxin in culture lysates of diverse ETEC strains (Paton et al., 2006). Similarly, a recombinant *L. casei* strain carrying the K88 fimbriae from ETEC was able to reduce the attachment of ETEC to porcine intestinal brush border in a dose-dependent manner and to reduce infection in a mice model (Wen et al., 2012). These studies demonstrate that bioengineered probiotics can be used in the targeted control of specific enteropathogens.

4.1.2 *Salmonella*

Salmonella infection is the leading cause of foodborne gastroenteritis in humans worldwide and is commonly associated with raw or uncooked poultry and eggs, and fruits and vegetables (Foley, Lynne, & Nayak, 2008; Weill et al., 2006). In the United States, nontyphoidal *Salmonella* is responsible for 11% of total foodborne illness and 35% of hospitalizations (Scallan et al., 2011). *Salmonella* utilizes numerous virulence factors to initiate infection and colonizes effectively on the epithelial cells in the gut (Ahmer & Gunn, 2011). Therefore, control strategies have to be applied along the food production chain to prevent the entry into human food supply (Vandeplas, Dubois Dauphin, Beckers, Thonart, & Théwis, 2010). Several studies have demonstrated that inoculation of cultures of one or several probiotic strains into broiler chickens may inhibit *Salmonella* contamination (Audisio, Oliver, & Apella, 2000; Higgins et al., 2007, 2008; Van Coillie et al., 2007). Neonatal broiler chicks were challenged with *Salmonella* Enteritidis and then challenged with *Lactobacillus* probiotic culture at different doses orally. *Lactobacillus* significantly reduced *Salmonella* incidence in chicks by 85% (Higgins et al., 2008). Similar study conducted in grower pigs challenged with *S. enterica* serotype Typhimurium and administered with *L. plantarum* resulted in a reduction in fecal shedding of the pathogen. In addition, probiotic feeding also improved the performance of the pigs (Gebru et al., 2010). *L. rhamnosus* was also able to reduce epithelial cells stress induced by heat or cytotoxicity induced by *S. Typhimurium* infection in a cultured epithelial cell model (Burkholder & Bhunia, 2009).

Commercially available probiotic cocktails were evaluated for their ability to inhibit *Salmonella* colonization in neonatal broiler chickens and turkey poult. Administration of probiotic cultures (FloraMax, IVS-Wynco LLC, Springdale, AR) significantly reduced *Salmonella* counts in the tonsils and ceca of chickens and poult (Menconi et al., 2011). Furthermore, administration of *Lactobacillus reuteri* strain that produced reuterin (bacteriocin) significantly reduced *Salmonella* populations and increased the survival rate in chicks (Zhang, Li, & Li, 2012). Probiotic *Bacillus subtilis* DSM 17299 was also able to significantly reduce cecal loads of *Salmonella* (Knap et al., 2011). An *in vitro* gut fermentation cellular model was used to evaluate the protective effect of probiotics against *Salmonella*. Addition of *Bacillus thermophilus* RBL67 to *Salmonella* in the reactors of the colonic fermentation model revealed a protective effect on epithelial integrity and increased the transepithelial resistance by 58% (Zihler, Gagnon, Chassard, & Lacroix, 2011). Probiotic has also been effective against antibiotic-resistant *S. Typhimurium* DT104 (Asahara et al., 2011). Administration of *L. casei* Shirota in mice challenged with *S. Typhimurium* significantly reduced the pathogen growth and subsequent extraintestinal dissemination. The increase in concentration of organic acids and lowering of pH in the intestine were thought to reduce probiotic colonization, which correlated with the antimicrobial activity (Asahara et al., 2011).

The mechanism underlying the antibacterial effect of *Lactobacillus* is multifactorial and involves lowering of the pH, production of lactic acid, and production of bacteriocins, nonbacteriocins, and nonlactic compounds (Dobson et al., 2012). It was observed that *L. johnsonii* La1, *L. rhamnosus* GG, *L. casei* Shirota YIT9029, *L. casei* DN-114001, and *L. rhamnosus* GR1 dramatically reduced the viability of *S. enterica* serovar Typhimurium through the production of nonlactic acid molecules, while the complete inhibition of *Salmonella* growth was observed to be due to a pH-lowering effect (Fayol-Messaoudi et al., 2005). In another study, soluble factors produced by *B. bifidum* also suppressed gene expressions in *S. Typhimurium* that are required for adhesion and invasion for systemic spread (Bayoumi & Griffiths, 2012). *In vivo* study using a mouse model demonstrated that continued administration of *L. casei* CRL diminished *Salmonella* counts in the intestine as well as its spread outside this organ. The probiotic-associated immunomodulatory effect involved both the innate and adaptive immune responses. Probiotic administration reduced neutrophil infiltration, activated phagocytic activity, increased IgA+ cells, and released sIgA specific to the pathogen in the intestinal fluid (de LeBlanc, Castillo, & Perdigon,

2010). Besides antimicrobial actions, probiotics also increased feed conversion and the performance in chickens and turkey poults.

4.1.3 *Campylobacter*

In poultry, *Campylobacter* is another predominant bacterial pathogen that is responsible for numerous outbreaks. Several probiotic strains have been evaluated for their efficacy in controlling *Campylobacter*. Human colon T84 and embryonic Int-407 epithelial cells were pretreated with *Lactobacillus* strains and then infected with *C. jejuni*. It was observed that *L. helveticus* R0052 reduced *C. jejuni* invasion into T84 cells and Int-407 cells by 35–41% and 55%, respectively. In addition, *L. helveticus* R0052 adhered efficiently to the epithelial cells suggesting that the inhibition of pathogen invasion could be due to competitive exclusion (Wine, Gareau, Johnson-Henry, & Sherman, 2009). In *in vivo* experiments conducted using a defined human microbiota-associated BALB/c mice were orally infected with either *C. jejuni* or *Salmonella* and then subsequently challenged with probiotic *Lactobacilli* and *Bifidobacteria*. Probiotics were able to enhance colonization resistance by successfully excluding both pathogens from mice and also increased proliferation of lymphocytes against *Salmonella* antigens. This study indicates that the probiotic administration reversed the immunosuppressive activity of *Salmonella* in BALB/c mice (Wagner, Johnson, & Rubin, 2009). Baffoni et al. (2012) evaluated the use of synbiotics to control *C. jejuni* in poultry. Prebiotic galacto-oligosaccharide was used with probiotic *B. longum* subsp. *longum* PCB133, and this synbiotic significantly reduced *C. jejuni* population in poultry feces thereby highlighting the positive effect of employing the synbiotic approach to reduce pathogen loads.

4.1.4 *L. monocytogenes*

Among the various pathogens causing foodborne illness, hospitalizations, and deaths, *Listeria* is responsible for 19% of the associated deaths (Scallan et al., 2011). Upon arrival in the gastrointestinal tract, *L. monocytogenes* invades the intestinal epithelium and disseminates from the mesenteric lymph nodes to the spleen and liver (Vazquez-Boland et al., 2001). During bacteremia, the organism reaches to liver, spleen, gall bladder, brain, and placenta. In the placenta, it can cause villous necrosis and microabscesses resulting in preterm abortion and infection of the fetus leading to stillbirth or neonatal listeriosis (Bakardjiev, Theriot, & Portnoy, 2006; Jiao et al., 2011). Several probiotics have been evaluated for their ability to control *L. monocytogenes* infection. dos Santos et al. (2011) evaluated the monoassociation of

L. delbrueckii in gnotobiotic mice and their effect on *Listeria* colonization. Administration of *L. delbrueckii* was capable of protecting the mice against death caused by *L. monocytogenes* and also led to a faster clearance of the bacteria from various organs such as the liver, spleen, peritoneal cavity, and the gut. Additionally, probiotic-fed mice also showed an increase in IFN- γ and IL-10 signifying the role for effector molecules in probiotic-induced cytoprotection. Similar results were obtained in a study where rats were fed with *L. casei* Shirota strain. In addition to reducing *Listeria* populations in the gut, spleen, liver, and feces, the probiotic strain also increased cellular immunity as determined by the delayed-type hypersensitivity response against heat-killed *L. monocytogenes* (de Waard, Garssen, Bokken, & Vos, 2002). Puertollano et al. (2008) evaluated the immunomodulatory effects of *L. plantarum* against *L. monocytogenes* infections in mice. Administration of *L. plantarum* in mice infected with *L. monocytogenes* resulted in a reduction in the production of proinflammatory cytokines which circumvented *Listeria*-mediated cytotoxicity. Several species of *Lactobacillus* and *Bifidobacterium* were also able to inhibit *L. monocytogenes* infection in a cell culture model (Corr, Gahan, & Hill, 2007). Later, the anti-infective property of probiotic *L. salivarius* was shown to be due to the production of a bacteriocin that protected mice from listeriosis when challenged with *L. monocytogenes* (Corr, Li, et al., 2007). In addition to the use of wild-type probiotics, Koo, Amalaradjou, and Bhunia (2012) generated a recombinant *L. paracasei* strain to control *L. monocytogenes* infection in a cell culture model. The recombinant probiotic was designed to express *Listeria* adhesion protein, an essential virulence factor (Burkholder & Bhunia, 2010; Jagadeesan et al., 2010) aiding *Listeria* in transepithelial translocation during intestinal phase of infection. Preexposure of intestinal monolayers to the recombinant probiotic followed by *Listeria* infection led to a reduction in adhesion and paracellular translocation by 44% and 46%, respectively. The recombinant probiotic also protected the monolayers from *Listeria*-mediated cytotoxicity and tight junction compromise. The use of such recombinant probiotics can help in the targeted elimination of enteric pathogens.

4.1.5 Miscellaneous bacterial pathogens

Probiotics have been evaluated for the control of *C. perfringens* in turkey poults (Rahimi, Kathariou, Grimes, & Siletzky, 2011). Administration of Primalac, a commercial probiotic cocktail to turkey poults, significantly reduced cecal *C. perfringens* counts compared to the control. In another

study, a recombinant *Pichia pastoris* containing *C. perfringens* alpha toxin gene significantly increased weight gain, feed efficiency, sero conversion, and an absence of adverse reactions in histopathological evaluation of broiler chicks (Gil de los Santos, Storch, Fernandes, & Gil-Turnes, 2012). Probiotic bacteria have also been used to control *Vibrio parahaemolyticus* attachment in cell culture model. *L. plantarum* AS1 was shown to attach efficiently to HT-29 cells and reduce *V. parahaemolyticus* attachment by competitive exclusion and displacement (Satish Kumar et al., 2011). A recombinant probiotic expressing a chimeric lipopolysaccharide was able to bind to cholera toxin and protected infant mice challenged with virulent *V. cholera* (Focareta, Paton, Morona, Cook, & Paton, 2006). Probiotics have also been shown to be effective against other pathogens such as *Shigella sonnei*, *S. aureus*, *E. faecalis*, *Proteus mirabilis*, and *P. aeruginosa* (Varma, Dinesh, Menon, & Biswas, 2010).

4.2. Probiotics to control viral pathogens

Probiotics have also been used to control viral infections. Gnotobiotic pigs fed with *L. acidophilus* and *L. reuteri* enhanced IFN- γ and IL-4 responses in serum and decreased human Rotavirus infection (Wen et al., 2009). Protection against rotavirus-induced diarrhea was also induced by administration of *L. paracasei* expressing variable domain of llama heavy-chain antibody fragments (Pant et al., 2006). Another major enteric virus responsible for 58% of foodborne illness is Norovirus (Marshall & Bruggink, 2011; Mattison, 2011). Probiotic-fermented milk containing *L. casei* Shirota strain has been evaluated for its efficacy in controlling norovirus gastroenteritis in a health service facility. A total of 77 people were enrolled in the study. Intake of probiotic-fermented milk by the treatment group resulted in a reduction in the mean duration of fever after the onset of gastroenteritis (Nagata et al., 2011). Wang, Yu, Gao, and Yang (2012) generated a recombinant *Lactobacillus* strain expressing the hemagglutinin of the avian influenza virus H5N1. Oral administration of this recombinant probiotic BALB/c mice triggered both mucosal and systemic immune responses. There was an increase in anti-HA IgA and anti-HA IgG levels with an associated increase in IL-4 production. Recombinant *L. casei* expressing human Lactoferrin was shown to exhibit antibacterial and antiviral activities in *in vitro* and *in vivo* models (Chen et al., 2010).

Receptor mimetics has been used as a strategy to control foodborne pathogens. Many enteric pathogens recognize oligosaccharides expressed on host cells as receptors for toxins and adhesion factors. This interaction between the ligand and the receptor is essential for the initiation of infection

process. This critical step in the infection process can be adapted to develop intervention strategies. This involves the expression of molecular mimics of host oligosaccharides in probiotic bacteria to control enteric pathogens (Paton, Morona, & Paton, 2010). Such designer probiotics can bind bacterial toxins to competitively inhibit pathogen binding to its receptor in the gut. Such receptor mimic probiotics have been developed against STEC and ETEC (Paton et al., 2005, 2006), Cholera (Focareta et al., 2006), and *C. jejuni* (Yuki et al., 2004). In addition to the enteric pathogens, probiotics have also been developed against many other pathogens and infectious agents summarized in Table 5.8.

4.3. Probiotics to neutralize toxins in food

Fungal pathogens could be controlled by inactivation of their toxins (mycotoxins) that cause foodborne infections. Mycotoxins are of clinical importance due to their ability to induce acute and chronic toxicity in the host (Kabak, Dobson, & Var, 2006). These mycotoxins can be mutagenic, carcinogenic, teratogenic, and immunosuppressive in nature (Salminen et al., 2010). Probiotics are known to bind and neutralize toxins. This attribute of the probiotic has been exploited in the reduction of dietary exposure to fungal toxins. Specific strains of probiotics have proved to be highly effective in removing mycotoxins in model systems. Armando et al. (2012) have demonstrated that *Saccharomyces cerevisiae* strains can adsorb ochratoxin and zearalenone in a mycotoxin binding assay under simulated gastrointestinal conditions. It was also observed that toxin binding was a function of cell wall thickness and binding occurs through physical adsorption. Similar studies conducted using a food system (grape juice) also demonstrated the ability of *Saccharomyces* strains to adsorb ochratoxin (Bejaoui, Mathieu, Taillandier, & Lebrihi, 2004).

Rats fed with aflatoxin-containing diet showed that administration of *Lactobacilli casei* and *L. reuteri* significantly reduced the oxidative stress induced by aflatoxins in comparison to the control group that did not receive any probiotics (Hathout et al., 2011). Similar experiment conducted in rats fed with different doses of aflatoxin (AFB-1) followed by administration with *L. reuteri* demonstrated significantly lower levels of AFB-1 in the intestine than in the control group. This study demonstrated that probiotic strains can bind to toxin in the intestine and act as a barrier to toxin-induced cell cytotoxicity (Hernandez-Mendoza, González-Córdova, Vallejo-Cordoba, & Garcia, 2011). Besides aflatoxin, ochratoxin, and zearalenone, other fungal toxins such as patulin and fumonisin can also be neutralized by probiotics.

Table 5.8 Lactic acid bacteria-based intervention strategies (vaccines)

Vaccine target	Vehicle	Antigen	Model	Effect	Reference
<i>Helicobacter pylori</i>	<i>L. plantarum</i>	Urease B	Mouse	Reduction in colonization	Corthesy, Boris, Isler, Grangette, and Mercenier (2005)
Tetanus	<i>Lactococcus lactis</i>	Tetanus toxin fragment C	Mouse	Survival after tetanus toxin challenge	Robinson et al. (2004)
<i>Streptococcus pneumoniae</i>	<i>L. plantarum</i> and <i>L. helveticus</i>	PsaA	Mouse	Reduction in nasal colonization	Oliveira et al. (2006)
ETEC	<i>L. acidophilus</i>	K99	Pig	Inhibition of adhesion	Tarahomjoo (2012)
SARS-associated coronavirus	<i>L. casei</i>	Spike antigen segments	Mouse	Viral neutralizing antibody elicited	Lee et al. (2006)
Rotavirus	<i>L. lactis</i>	VP7	Mouse	Neutralizing antibody against VP7	Perez, Eichwald, Burrone, and de Mendoza (2005)
Group B Streptococcus	<i>L. lactis</i>	Pilus	Mouse	Survival of offspring from vaccinated mothers after infectious challenge	Buccato et al. (2006)

Adapted from [Wells and Mercenier \(2008\)](#).

Several probiotic strains such as *Propionibacterium*, *L. rhamnosus* GG, *L. plantarum*, *L. casei* Bacillus species, and *E. faecium* were demonstrated to bind and neutralize mycotoxins (Danicke & Doll, 2010; Hernandez-Mendoza, Garcia, & Steele, 2009; Hernandez-Mendoza et al., 2011; Khanafari, Soudi, Miraboulfathi, & Osboo, 2007; Niderkorn, Morgavi, Aboab, Lemaire, & Boudra, 2009; Topcu, Bulat, Wishah, & Boyaci, 2010).



5. DELIVERY SYSTEM FOR PROBIOTICS TO THE GUT

When using probiotics for control of infections, it is essential that they survive the acidic gastric pH, the intestinal enzymes, bile, and alkaline pH to be able to exert their protective effect. This is critical when using recombinant probiotic to ensure production of the foreign protein at the target site. In general, probiotic supplements have been delivered as capsules, tablets, or powders. The choice of delivery matrix is also important to ensure probiotics activity and viability (Hajela, Nair, Abraham, & Ganguly, 2012).

Probiotic encapsulation technology (PET) has emerged as the principal method for delivery of probiotics. A wide range of microbes have been immobilized using semipermeable and biocompatible materials that modulate their delivery and release. Probiotics are either entrapped or encapsulated. Entrapment refers to the trapping of material within or throughout a matrix. Encapsulation involves the formation of a continuous coating around the inner matrix that is present within the capsule wall as a core of the encapsulated material. Encapsulation helps to stabilize cells, enhancing their viability and stability during production, storage, and handling. PET helps in the controlled and continuous delivery of probiotic cells in the gut. The biomaterials used in the encapsulation of probiotics include alginate, carrageenan, gelatin, chitosan, whey proteins, cellulose acetate phthalate, locust bean gum, and starches (Anal & Singh, 2007; Chandramouli, Kailasapathy, Peiris, & Jones, 2004; Krasaekoopt, Bhandari, & Deeth, 2003).

Alginate gels are insoluble in acidic media and hence protect probiotics from gastric acidity (Gbassi, Vandamme, Ennahar, & Marchioni, 2009). Carrageenan is used for its ability to form gels that can entrap the probiotics bacteria (Doleyres, Fliss, & Lacroix, 2004; Doleyres, Paquin, LeRoy, & Lacroix, 2002). Cellulose acetate phthalate is insoluble at pH below 5 but soluble when pH is greater than 6. This is especially advantageous for the stability of encapsulated probiotic in the stomach and for controlled release in the intestine (Anal & Singh, 2007). Microencapsulation of probiotics is performed by freeze-drying, extrusion, or emulsification.

Freeze-drying results in the formation of concentrated dry powder that has longer shelf life but do not protect probiotics from gastric fluid or bile (Rokka & Rantamaki, 2010). It is essential to provide probiotics with physical barriers to protect them from the adverse conditions encountered in the gut. For this purpose, extrusion and emulsification techniques intended to develop gel beads or capsules were applied (Lacroix, Paquin, & Arnaud, 1990). Extrusion technique involves the mixture of probiotics with the hydrocolloid finally resulting in the formation of gelled droplets called beads (Gouin, 2004). Emulsification involves the mixture of probiotics with a continuous phase resulting in a water-in-oil emulsion. This technique has been used to develop encapsulated probiotics (Shima, Morita, Yamashita, & Adachi, 2006). Emulsification results in the formation of oily or aqueous droplets called capsules (Gbassi & Vandamme, 2012).

Alginate microcapsules coated with chitosan were evaluated for their ability to increase gastrointestinal viability of *B. breve*. Chitosan stabilizes the alginate microcapsules at pH above 3. Coating with chitosan also increased the survival of *B. breve* in simulated gastric fluid and prolonged its release upon exposure to intestinal pH (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2011). Calinescu and Mateescu (2008) developed a tablet dosage system based on carboxylated methyl high amylose starch (CM-HAS) and chitosan excipient for probiotic colon delivery. CM-HAS ensures protection in the gastric pH while chitosan helps with slow release of the probiotic in the intestine. This encapsulation of *L. rhamnosus* resulted in an improved percentage of delivered bacteria in simulated intestinal conditions. To enhance the slow and sustained release of probiotics in the gut, a coacervate extended-release microparticulate delivery system was developed (Alli, 2011). Core mucoadhesive *L. rhamnosus* was prepared using hypermellose and subsequent enteric coating with hypermellose phthalate. Hypermellose has good mucoadhesive and release rate controlling properties, which are preferred in mucoadhesive formulations. Use of such microparticles in simulated intestinal conditions exhibited appreciable mucoadhesion compared to the freeze-dried cultures.

In addition to the use of the above-mentioned matrices, DNA-based gels have also been evaluated as a vehicle for oral delivery of probiotics. DNA-based gels were initially prepared to protect lactic acid bacteria from extreme conditions in commercial yogurts (Jonganurakkun, Liu, Nodasaka, Nomizu, & Nishi, 2003; Jonganurakkun, Nodasaka, Sakairi, & Nishi, 2006). The DNA forms complexes with gelling agents such as gelatin and

κ -carrageenan at low pH. This resulted in the formation of two kinds of gel: hydrogels and complex gels. In a hydrogel, the bacteria are fixed in the gel when the yogurt containing bacteria is added to the precooled mixture of DNA and gelling agent. In a complex gel, a mixture of yogurt and cacao oil is added to the gel solution containing the DNA and the gelling agent to form the encapsulated probiotic. It was seen that complex gel was able to protect the probiotic from adverse simulated gastric conditions. Hydrogels in addition to protecting from acidic conditions were also more stable during 4 °C storage which corresponds to the storage temperature at which the product would be maintained for prolonged storage (Jonganurakkun et al., 2006). Thus, DNA-based hydrogels offer a potential delivery system for oral administration of probiotics.



6. CONCLUSION AND FUTURE PERSPECTIVES

Probiotics have been extensively studied in *in vitro* and *in vivo* models. Ample evidence is documented to support the potential application of probiotics for the prevention and treatment of enteric infections. Health benefits of probiotics include the prevention of diarrhea, atopic eczema, antibiotic-associated diarrhea, traveler's diarrhea, prevention of dental carries, colorectal cancers, and treatment of IBD. However, as with any potential intervention strategies, probiotics also have a safety concern. Therefore, it is essential that toxic and metabolic effects of probiotics on humans are assessed for patient safety, especially for critically ill or immunocompromised patients. Significant challenges still exist in the effective application of probiotics in pathogen control. Future studies are essential to define optimal doses and their correct combinations of various probiotic species based on their molecular mode of antimicrobial action. There is also a need for improvement of production techniques to understand and develop better approaches to probiotic delivery and bioavailability in the gut as we move from theoretical benefits to clinical application. Now with the possibility to express different molecules in probiotic bacteria, such as enzymes, cytokines, receptor mimics, adhesion molecules, antibodies, and host targeting molecules, future research can help optimize applications and develop biologically contained strains to support clinical trials. Thus, probiotic bacteria can be used to control and prevent pathogen colonization in the food animals to improve food safety and a realistic therapeutic option in humans to control enteric pathogens.

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