1 Probiotics and Health: From History to Future

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1.1 EARLY HISTORY OF THE USE OF MICROORGANISMS FOR HUMAN BENEFIT

There is evidence from wall carvings that cultured milk products were made at least 4500 years ago. Written evidence for fermented milks appears in Genesis 18: 8, “He then brought some curds and milk that had been prepared and set these before them”. The production of wine is referred to in Genesis 9: 20, “Noah a man of the soil, proceeded to plant a vineyard, where he drank some of its wine, he became drunk and lay uncovered inside his tent”. In Exodus 12: 39 the use of microorganisms to prepare bread is cited: “They baked the dough which they had brought out of Egypt into cakes of unleavened bread. For it had not become leavened, since they were driven out of Egypt and could not delay.” The exodus from Egypt is believed to have occurred approximately in 1440 BC. Homer in the Iliad, written between 900 and 800 BC makes numerous references to wine and cheese. In book 11 of the Iliad there is the following passage: “Pours a large portion of Pramnian wine; with goats milk cheese a flavourous taste bestows, and last with flour the smiling surface stows”.

The ancient production of wine, cheese and bread served a number of useful purposes. It altered the flavor and texture of the natural foods and in the case of milk products extended the time of edible use by preventing rapid spoilage by random bacterial or fungal growth. In the case of wine, in addition to its pleasurable mind-altering properties, wine was used as an anesthetic. In a 10th-century Persian work, the Shahnameh, the use of wine is described for performing Caesarean sections. In India wine was used as an anesthetic by the surgeon Sushruta around 600 BC. Therefore a long history exists for the use of microorganisms to benefit the human condition.

In more recent times an early reference to the use of microorganisms for a specific medical condition was proposed by Doderlein (1892), in which year he proposed to treat vaginal infections with lactobacilli. In 1900 Henry Tissier at the Pasteur Institute isolated a Bifidobacterium from a breast-fed infant (Tissier, 1905). This bacterium is now designated Bifidobacterium bifidus. Tissier also showed that bifidobacteria are the predominant organism found in breast-fed infant feces and recommended administering this organism to infants with diarrhea. In 1907 the use of a specific class of microorganisms to benefit human health was introduced to the general public by the Nobel Prize winner Elie Metchnikoff. In his book The Prolongation of Life (1907), Metchnikoff stated his belief
that bacteria in the colon were responsible for adverse health in adults and that consuming sour milk or yogurt would counteract these harmful bacteria. He proposed that the strain “Bulgaricus Bacillus”, later named \textit{Lactobacillus bulgaricus}, was the strain responsible for conferring better health and longer life in humans. In 1911 Douglas published \textit{The Bacillus of Long Life}, which supported the concept of human longevity and the consumption of fermented milk. In 1917 Alfred Nissle isolated an \textit{Escherichia coli} that he used to treat acute intestinal diseases such as salmonellosis and shigellosis, with a significant success rate. This organism is now designated \textit{E. coli} Nissle and is still used as a probiotic and is an example of a non-lactic acid bacteria probiotic. In 1935, Retteger at Yale University proposed that \textit{Lactobacillus acidophilus} would be an appropriate species to use for human clinical trials (Retteger \textit{et al.}, 1935). This approach was followed by a study demonstrating positive results for patients with chronic constipation. The use of specific bacteria for human disorders dates to the 1920s but the term “probiotic” was not used in this context until 1974. Parker (1974) described probiotics as “organisms and substances, which contribute to intestinal microbial balance”. In 2002 a European Expert Committee (FAO, 2006) defined probiotics as “living microorganisms, which upon ingestion in adequate amounts exert health benefits beyond inherent general nutrition”.

### 1.2 Overview of Probiotic Studies and Results for the Past 35 Years

Based on the definitions for a probiotic expressed in 1974 and modified in 2002, a significant number of microorganisms have been isolated and identified as probiotics. Some of these probiotics have been fed to humans and animals to test, treat or prevent various diseases, disorders and syndromes. The approximate number of different bacterial strains in each genera that have been attributed as probiotics are as follows: \textit{Lactobacillus}, 23; \textit{Bifidobacterium}, 5; \textit{E. coli}, 2; and one strain each of \textit{Bacillus}, \textit{Streptococcus}, \textit{Enterococcus} and \textit{Lactococcus}. In addition there is one yeast, namely \textit{Saccharomyces boulardii}, that has probiotic attributes (Sanders, 2007). The list is increasing yearly and as will be discussed later in this chapter propionibacteria will most certainly be added to the list of genera. With the corresponding isolation and identification of probiotic microorganisms there has been an increasing number of basic research, clinical research, clinical trial and intervention studies published.

Year to year, since the mid-1980s, the number of papers has increased exponentially. It will not be possible in a chapter or a book to cover all the studies in print and therefore the following sections describe the highlights of the findings on health benefits.

### 1.3 Current Evidence for Probiotic Health Benefits

#### 1.3.1 Lactose Intolerance

Worldwide many millions of people experience lactose malabsorption. The frequency of the disorder increases with age. The cause for this disorder is a decline in the activity of the enzyme lactase in the intestinal brush border mucosa. This decline in activity results in lactose malabsorption. This incomplete absorption causes flatus, bloating, abdominal
cramps, and moderate to severe diarrhea. A major consequence of this sequence of events is a severe limitation in consumption of dairy products, which is particularly pronounced in the elderly. Several studies have demonstrated that during the fermentation of milk to make yogurt lactase is produced and on consumption of yogurt this lactase is active in the intestinal tract (Kim & Gilliland, 1983; Kolars et al., 1984; Savaiano et al., 1984; de Vrese et al., 2001). The organisms used for the production of yogurt are Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus. Kim and Gilliland (1983) found that feeding yogurt to participants who were lactose-intolerant caused a significant reduction in the levels of hydrogen found in the breath compared with feeding milk to subjects with the same condition. The level of hydrogen in the breath reflects the intestinal microflora metabolism of lactose not absorbed in the small intestine and thus present in the colon, where the microflora are present in high concentrations. Kolars et al., (1984) found that subjects who ingested 18 g of lactose in yogurt had 67% less hydrogen in their breath compared with the same lactose dose delivered in milk. An analysis of intestinal duodenal aspirates obtained from the subjects consuming yogurt indicated that there were significant levels of lactase in the duodenum. A systematic review of the published literature in 2005 analysing studies of probiotic treatment of adult lactose intolerance concluded that the evidence does not support the effectiveness of probiotics for treatment of this disorder (Levri et al., 2005). However, the authors conclude that this may result from the variation in the nature or type of probiotic used in the specific study. For example, lactobacilli that have low levels of lactase could be potential confounder. The strains selected for yogurt production have high lactase levels, required for the efficient preparation of yogurt.

1.3.2 Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a major medical problem. IBD is a general term used for intestinal inflammation, and the specific diseases and disorders that fall into the IBD category include Crohn’s disease, ulcerative colitis, and irritable bowel syndrome. One of the important potential medical applications for probiotics is the treatment and prevention of IBD relapses. There have been a limited number of reports of the beneficial effects of probiotics in treating or alleviating IBD symptoms. It has been shown that E. coli Nissle is helpful in maintaining the remission phase for patients with Crohn’s disease (Malchow, 1997). Administration of Lactobacillus salivarius in milk to interleukin (IL)-10 knockout mice significantly reduced inflammation in the cecum and colon compared with the same knockouts fed milk alone (O’Mahoney et al., 2001). IL-10 is an anti-inflammatory cytokine that causes progressive colonic inflammation when levels are low or absent, as is the case for these knockout mice. These results suggest that probiotics, alone or by interaction with the existing intestinal flora, can influence the colonic immune system and counteract low IL-10 levels. IL-10 is normally expressed in T cells in the lamina propria of the colon. In another murine model study, IL-10 knockout mice treated with a combination of L. salivarius and Bifidobacterium longum subsp. infantis in a dairy product resulted in a decrease in disease severity. The severity of disease was evaluated by weight loss, colon pathology and general appearance over a 6-week period (McCarthy et al., 2003). Control animals fed only dairy products exhibited a chronic wasting disease during the same time period. VSL#3 is a product containing multiple probiotic strains (Sheil et al., 2007). VAL#3 was tested in patients with ulcerative colitis; 15 of 20 patients treated remained in remission over the 12-month period of the study, suggesting the mixture may be useful in maintaining remission in patients with ulcerative colitis (Venturi et al., 1999). A study involving 32 patients
with Crohn’s disease in clinical remission and given either mesalamine or mesalamine plus *S. boulardii* showed that 37% of patients given the drug alone relapsed in 6 months while 6.5% of patients receiving drug plus *S. boulardii* relapsed (Guslandi *et al*., 2000). These data suggest that *S. boulardii* could be a useful adjuvant for preventing symptomatic relapse in patients with Crohn’s disease. The sum total of the existing human and animal probiotic IBD literature is preliminary and equivocal; however, it does suggest that specific probiotics could be useful in preventing symptomatic relapse for patients with ulcerative colitis and/or Crohn’s disease.

### 1.3.3 Treatment of gastroenteritis

The most extensive probiotic medical literature is in the area of diarrheal diseases (gastroenteritis). The treatment and prevention can be further categorized by etiologic agent or by the type of disease.

#### 1.3.3.1 Antibiotic-associated diarrhea

There have been numerous studies investigating the efficacy of probiotics for preventing or reducing the frequency and severity of diarrhea associated with the clinical use of antibiotics (Siitonen *et al*., 1990; Arvola *et al*., 1995; Vanderhoof *et al*., 1999; Armuzzi *et al*., 2001a,b; Cremonini *et al*., 2002). When studying 119 children who received antibiotics for respiratory infections and concomitant *Lactobacillus rhamnosus* GG (LGG) or placebo during the antibiotic treatment period, investigators found a 70% reduction in diarrheal symptoms for the group administered LGG compared with a placebo arm (Arvola *et al*., 1995). In a larger study involving 202 children treated with oral antibiotics, 8% of the children who were given LGG concurrently with antibiotic experienced diarrheal symptoms compared with 26% of the placebo group (Vanderhoof *et al*., 1999). In two studies with 60 and 120 adult patients respectively receiving antibiotic treatment to eliminate a *Helicobacter pylori* infection, investigators found that a significantly lower number of patients who received concurrent LGG experienced nausea and diarrhea compared with a group given placebo (Armuzzi *et al*., 2001a,b). *Helicobacter pylori* has been identified as an etiologic agent for gastric ulcers. *Saccharomyces boulardii* has also been shown to reduce antibiotic-associated diarrhea (Marchand & Vandenplas, 2000). A meta-analysis of the effect of probiotic administration on antibiotic-associated diarrhea comprising 22 placebo-controlled studies found a combined relative risk of 0.39 for diarrhea among the probiotic-treated cohorts (D’Soriza *et al*., 2002). The investigators concluded that a strong benefit exists for probiotic administration for antibiotic-associated diarrhea, although they cautioned that the evidence is not yet definitive and more studies are required.

#### 1.3.3.2 Acute diarrhea

Numerous studies have reported the use of probiotics to prevent or treat acute diarrhea (Hochtes *et al*., 1990; Cetina-Savri & Sierra, 1994; Raza *et al*., 1995; Sepp *et al*., 1995; Pant *et al*., 1996; Shornikova *et al*., 1997a,b; Oberhelman *et al*., 1999; Guandalini *et al*., 2000; Mastretta *et al*., 2002; Szajewska *et al*., 2001; Allen *et al*., 2003). The majority of the studies involved infants or children and the etiologic agent was rotavirus or of unknown cause. Probiotics that have been shown to be effective for the treatment of acute gastroenteritis include LGG, *Lactobacillus reuteri* and *S. boulardii* (Hochtes *et al*., 1990;
Cetina-Savri & Sierra, 1994; Raza et al., 1995; Sepp et al., 1995; Pant et al., 1996; Shornikova et al., 1997a,b; Oberhelman et al., 1999; Guandalini et al., 2000; Mastretta et al., 2002; Szajewska et al., 2001; Allen et al., 2003). A multicenter European based trial with 287 children aged 1–36 months from 10 countries is one of the most extensive trials investigating probiotic treatment for acute diarrhea reported (Guandalini et al., 2000). The children were experiencing moderate to severe diarrhea. The patients were randomized to be given placebo or LGG along with oral rehydration solution. The children receiving LGG had a shorter duration and decreased severity of disease along with a shorter hospital stay. Another important finding was that on follow-up the probiotic-treated children had a decreased likelihood of persistent diarrheal illness. There are other examples of findings similar to those described above in children with diarrheal disease (Pant et al., 1996; Shornikova et al., 1997b). A review of the double-blind randomized literature for probiotic biotherapeutic agents found that LGG and *S. boulardii* had the most favorable effect for treatment of acute diarrhea in children and adults (Marchand & Vandenplas, 2000).

### 1.3.3.3 Traveler’s diarrhea

Visitors from countries with temperate climates to areas with tropical or subtropical climates experience a high incidence of diarrhea. The incidence rate often approaches 50%. There have been a few published studies that have investigated the efficacy of probiotic treatment for lowering the diarrheal incidence rate. A study that tracked Finnish travelers to Turkey showed that in one of two resorts oral ingestion of LGG conferred a significant protection rate of 30.5% and 27.9% in weeks 1 and 2 of the study respectively (Oksanen et al., 1990). In another study, 245 travelers from New York were followed for 1–3 weeks after arriving in various developing countries. The travelers were provided with LGG or a placebo prior to their trip and LGG afforded a protection rate of 47% (Hilton et al., 1997).

McFarland (2007) performed a meta-analysis of studies designed to investigate probiotics for the prevention of traveler’s diarrhea. The analysis included 12 studies that met the inclusion and exclusion criteria. The results of the analysis showed that the pooled relative risk was 0.85 (*P* < 0.001) and that probiotics significantly prevent traveler’s diarrhea. The meta-analysis investigator also concluded that *S. boulardii* and a mixture of *L. acidophilus* and *Bifidobacterium bifidum* had significant treatment efficacy.

### 1.3.3.4 Treatment of relapsing gastroenteritis caused by *Clostridium difficile* toxin

Often as a result of antibiotic treatment, the normal intestinal microflora can be altered. The disturbance to the microflora can result in *C. difficile* growth from existing spores, with the concomitant production of toxin in the intestinal tract. Several studies have shown that treatment with LGG prevents relapse of gastroenteritis (i.e. recurrent *C. difficile*-associated disease, RCDAD) after use of antibiotics. Clinical observations have indicated a 60% relapse rate after therapy with metronidazole or vancomycin. Only 16% who had received LGG had a relapse and after a second course of treatment with LGG, there was a 94% overall cure rate (Gorbach et al., 1987; Biller et al., 1995; Bennet et al., 1996). There have been several recent studies that have cast doubt on these earlier findings. No benefit was found for a yogurt/LGG formulation for patients with RCDAD (Pochapin, 2000). In a small study using capsules containing lyophilized LGG there again was no benefit noted
with the probiotic, although the study had too few subjects to provide statistical power (Lawrence et al., 2005). It is therefore not clear if probiotics are beneficial for patients with RCDAD.

### 1.3.4 Cholesterol lowering

There is some evidence based on human studies that probiotics may lower total serum cholesterol and/or low-density lipoprotein (LDL) cholesterol. The results are not definitive and often conflicting. The lowering of LDL cholesterol would have important implications for decreasing the risk of coronary artery disease and for fatal myocardial infarction. The human studies that have shown an effect for fermented milk products on plasma cholesterol levels found a lowering of total cholesterol between 5.4 and 23.2% and for LDL between 9 and 9.8% (Anderson & Gilliland, 1999). A recent study of 14 subjects in a randomized crossover trial involving ordinary yogurt or yogurt plus *L. acidophilus* and *B. animalis* subsp. *lactis* for 6-week feeding periods and a 4-week washout period found a significant decline in serum total cholesterol when comparing the yogurt plus probiotics to the yogurt alone (Atoie-Jafari et al., 2009). The cholesterol studies have had small numbers of subjects and were limited in duration, generally 6 weeks. Based on *in vitro* and animal studies, several mechanisms for the probiotic lowering of serum cholesterol have been proposed. These involve absorption or assimilation of cholesterol by probiotics (Walker & Gilliland, 1993). There has been a study showing optimal removal of cholesterol from growth media in the presence of *L. casei* plus a prebiotic (Liong & Shah, 2005). A separate mechanism that has been proposed for probiotic-induced cholesterol lowering is the ability of bifidobacteria and lactobacilli to deconjugate bile acids. The deconjugation would lead to more rapid excretion of bile acids in the feces and since cholesterol is a precursor for bile acid synthesis, the lower bile acid concentration would act as a positive feedback for increasing synthesis from cholesterol to bile acids (Walker & Gilliland, 1993).

### 1.3.5 Treatment for urogenital infections

Vaginal infections are caused by such agents as *Candida*, *Trichomonas*, or bacterial organisms such as *Gardnerella vaginalis* and *Mycoplasma hominis*. Urinary tract infections are far more common in women and are generally caused by *E. coli*, *Chlamydia* and *Candida*. There are approximately 300 million urogenital infections reported per year. Normal healthy women have approximately 50 different species of microorganisms in the vaginal flora. Reid et al. (1995) reported that weekly intravaginal instillation of lactobacilli in 10 premenopausal women reduced urinary tract infections from 6.3 per patient per year before treatment to 1.3 per patient per year during treatment. Hilton et al. (1992) found that yogurt containing *L. acidophilus* reduced *Candida*-caused vaginitis by threefold in a crossover-designed trial. The results of studies using probiotics for treatment or prevention of urogenital infections are very limited, although there are investigators attempting to design specific probiotics to be administered orally to prevent or reduce the incidence of urogenital infections.

### 1.3.6 Treatment of allergic reactions

The most extensive studies directed at probiotic modulation of the immune response to food allergens has been done with LGG for preventing and treating atopic eczema. In a study of 159 pregnant women with a family history of atopic disease the subjects were
given either LGG or placebo for 2–4 weeks prior to their expected delivery (Kalliomaki et al., 2001). Women who breast-fed their infants received LGG or placebo for 6 months and women who bottle-fed their newborns fed them LGG or placebo for 6 months. A 50% reduction in the incidence of atopic eczema was noted in the first 2 years of the child’s life for the group receiving LGG compared with the placebo group. In a follow-up to this study, after 4 years the children given LGG had a significantly lower incidence of atopic eczema compared with the placebo group (Kalliomaki et al., 2003). In another study 27 infants with atopic eczema were randomized into three groups and given LGG, *Bifidobacterium animalis* subsp. *lactis* or placebo (Isolauri et al., 2000). After 2 months the clinical score for the severity and extent of the eczema indicated a significant improvement in the skin condition of the infants fed the probiotics (*P* = 0.002). A similar study in which 31 infants with atopic eczema had their exposure to cows’ milk terminated and were treated with LGG showed a significant improvement compared with a group who were not fed cows’ milk and were fed placebo (Majamaa & Isolauri, 1997). *Bifidobacterium animalis* has also been shown to reduce the severity of atopic eczema in young children (Majamaa & Isolauri, 1997).

### 1.3.7 Prevention of dental caries

After oral ingestion, probiotics can be isolated from the oral cavity. Therefore it would be logical to study their efficacy in preventing dental caries. In addition, LGG has been shown to have antimicrobial activity against the *Streptococcus* spp., an organism involved in causing tooth decay (Silva et al., 1987). Children in a multicenter daycare trial were given LGG-containing milk or non-supplemented milk and examined before and after the 7-month intervention study. The children receiving the probiotic had a lower rate of clinical development of dental caries, which was most pronounced in the group aged 3–4 years (Nase et al., 2001). More studies are needed to see if this observation can be repeated and if other probiotics will have the same beneficial effect.

### 1.3.8 Treatment and prevention of cancer by probiotics

By virtue of their metabolic activity, probiotics can influence the etiology of colon cancer and possibly tumors at other sites. Probiotics have been shown to reduce intestinal bacterial enzymes involved in the activation of procarcinogens (Hosoda et al., 1996). Probiotics also can produce short-chain fatty acids that may also be protective in the colon. Animal studies in rats have shown that probiotics can inhibit the formation of aberrant crypt foci in the colon. A combination of inulin plus *B. longum* reduced chemically induced aberrant crypt foci by 74% (Rowland et al., 1998). Inulin alone reduced the aberrant crypts by 21%. Rats fed a mixture of oligofructose, inulin, LGG and *B. animalis* subsp. *lactis* had significantly lower azoxymethane-induced colon tumors (Marotta et al., 2003). Mice genetically bred to be susceptible to colitis and colon cancer had a 10% incidence rate of adenocarcinoma when fed *L. salivarius* compared with the 50% rate for control animals (O’Mahoney et al., 2001). Rats injected with DMH and fed LGG had a significantly lower colon cancer incidence than animals receiving DMH alone (Goldin et al., 1996). Human colon cancer trials have not been conducted with probiotics, primarily due to the difficulty of conducting a preventive intervention trial. There is one report in the literature of a human trial of patients with superficial bladder cancer. The patients receiving *L. casei* had an 80% longer disease-free period, with a mean of 350 days compared with 195 days for the control group.
1.3.9 Additiona l health benefits attributed to probiotics

There are a number of other health benefits that have been observed for probiotic use over the past number of years. Some are included in this section. A study conducted in Italy with children suffering from cystic fibrosis and given LGG for the chronic abdominal pain often associated with the disease indicated that the frequency and severity of abdominal problems were reduced and that intestinal inflammation as judged by the fecal marker calprotectin and rectal nitric oxide was also decreased (Bruzzone et al., 2004). Rheumatoid arthritis is a systemic inflammatory disease. Animal studies using experimental arthritis model in Lewis rats showed that these rats improved when fed LGG compared with placebo (Baharav et al., 2004). The findings of a preliminary study involving 21 patients with rheumatoid arthritis receiving either placebo or LGG showed that the LGG group had a decreased number of swollen joints and lower overall arthritic activity, although the difference did not reach statistical significance (Hatakka et al., 2003). Nanji et al. (2005) studied the ability of probiotics to prevent alcohol-induced liver disease in a rat model. Rats were conditioned to drink ethanol and one group was administered LGG orally. The rats fed LGG had reduced liver disease and lower plasma endotoxin levels. In a related study rats were given carbon tetrachloride to induce chronic liver disease as a model to study the efficacy of probiotics in spontaneous bacterial peritonitis (Bauer et al., 2002). LGG was not effective and did not prevent bacterial overgrowth or bacterial translocation from the colon into mesenteric lymph nodes or portal blood.

The effect of probiotics on radiation exposure has been studied in a mouse model (Dong et al., 1987). Mice were either fed LGG or maintained on a normal diet and then exposed to 14 Gy of total body irradiation. The LGG-fed rats had a significantly lower mortality rate at 48 hours after irradiation. Of the 21 control mice 10 had Pseudomonas aeruginosa bacteremia, compared with 1 of 21 mice fed LGG. None of the LGG-fed mice had LGG bacteremia. There is a preliminary study from Japan using a streptozotocin-induced diabetic mouse model which showed that feeding LGG lowered hemoglobin A1c blood levels and improved glucose tolerance compared with controls (Tabuchi et al., 2003). Bone marrow transplantation patients can develop graft-versus-host disease (GVHD). Bacterial lipopolysaccharide (LPS) is believed to be involved in this process. A mouse model of GVHD has been developed where the disease is induced by employing a major histocompatibility mismatch (Gerbitz et al., 2004). The animals show serious damage to the bowel mucosa and high levels of serum LPS and inflammatory cytokines. The animals were divided into three groups, receiving in their drinking water LGG, ciprofloxacin or no additive for 7 days prior to transplantation. Treatment with LGG reduced mortality, which was most prominent in the early post-transplantation period and was reflected in a lower GVHD score compared with the other groups. Mesenteric lymph nodes of LGG treated animals had a lower concentration of translocated intestinal organisms.

1.3.10 Conclusions based on past and present use of probiotics for health applications

This section has outlined the current knowledge regarding the application of probiotics for preventing and treating medical diseases and disorders. Table 1.1 lists the medical applications for probiotics that have been studied in the past and which are currently under investigation.
There are numerous reports showing that probiotics can influence nutritional status. Bifidobacteria have been shown to produce the water-soluble vitamins thiamine, nicotinic acid, folic acid, pyridoxine, biotin and B₁₂ (Lee et al., 1999). Additional nutritional effects have been noted for L. acidophilus, which increases iron bioavailability (Lee et al., 1999), and numerous lactobacilli species deconjugate bile acids (Walker & Gilliland, 1993). As noted earlier probiotics can hydrolyse lactose in milk products.

### 1.5 FUTURE DEVELOPMENT AND USES OF PROBIOTICS FOR HEALTH APPLICATION

Sections 1.1–1.4 have reviewed the past and current development and applications of probiotics for nutrition and health purposes. In this section a review of possible new probiotic development and uses will be discussed. The future is always harder to evaluate than the past.
past; however, with current probiotic projects and goals in mind, this section will attempt to predict the future for probiotics.

The major thrusts in future health applications for probiotics will be based on the development of new organisms, through genetic modification (GM) or by natural selection, that specifically exhibit activities that would, from a mechanistic approach, apply to specific diseases, disorders or nutritional or drug requirements. The capability to achieve this objective will define current uses and additional future applications for probiotics. This section will not attempt to cover the area of bacterial genetics or techniques for gene insertion and current knowledge of bacterial genomics. Given current progress, this area would require at a minimum an entire book or possibly a multivolume series of books.

1.5.1 Probiotics as a platform for delivery of drugs, enzymes, hormones, nutrients and micronutrients

One of the intriguing areas of current research and future development of probiotics is their use as delivery systems for health-related compounds, enzymes, toxin inhibitors, carcinogen detoxifiers and immune modulators. Lothar Steidler has developed one of the best examples of using GM probiotics to deliver anti-inflammatory agents to the colon. A strain of *Lactococcus lactis* has been modified to express murine and human IL-10, a potent anti-inflammatory cytokine (Steidler et al., 2003). Knockout IL-10 mice rapidly develop colonic inflammation and subsequently adenocarcinomas (Scheinin et al., 2003). When introduced orally the recombinant *L. lactis* has been shown to have a positive effect by reducing intestinal inflammation in mice treated with colitis-inducing dextran sulfate (Steidler et al., 2000). These investigators have succeeded in replacing the thymidylate synthetase gene with the human IL-10 gene. This replacement results in a probiotic that can produce human IL-10 but which is not capable of synthesizing thymidine. The inability to make thymidine assists in biocontainment of the mutant, since the *L. lactis* now requires thymidine for growth and would not thrive in an outside environment. The GM *L. lactis* have been fed to a small number of patients with Crohn’s disease in a Phase I human clinical trial (Braat et al., 2006). This type of GM probiotic is a model for future development of organisms that can decrease local inflammation at the site where the probiotic resides. Probiotics with IL-4 or IL-12 producing genes can expand the array of organisms to combat colon inflammation and treat IBD and possible lower the subsequent risk for developing adenocarcinomas. Future directions for probiotics could include insertion of higher plant genes responsible for multistep synthesis pathways leading to anti-inflammatory products. An example would be curcumin or flavonoids and their analogues, which have been shown to be beneficial in treating inflammatory disease and dermatological disorders. These compounds are believed to act through inhibition of inducible nitric oxide synthetase. Recombinant bacteria with herb or plant genes directed toward the synthesis of a variety of medicinal products can have great potential for future probiotic development. This approach is dependent on methods of biocontainment that would prevent survival of the organism outside the human or animal and this should act to prevent environmental contamination.

1.5.2 Toxin sequestration

Studies have shown that various strains of *Lactobacillus* and *Bifidobacterium* have the ability to bind and inactivate aflatoxins (Gratz et al., 2005). Investigators have constructed strains of GM *E. coli* that can bind Shiga toxin (STX) produced by toxigenic *E. coli* or by *Shigella dysenteriae* (Paton et al., 2001). The possibility of selecting
probiotics that can bind a variety of bacterial toxins, either naturally (as for aflatoxin) or by GM (as for STX) is feasible in the future.

### 1.5.3 Carcinogen detoxification

A major challenge for understanding the causes of human cancers is identifying dietary or environmental agents involved in the etiology of cancer at different organ sites. Most agents are believed to be procarcinogens that require enzymatic or other types of catalysis to generate the direct-acting carcinogens. Therefore inhibitors of the activating enzymes that convert procarcinogens to carcinogens or the introduction of enzymes that deactivate the direct-acting carcinogens can interfere with chemical carcinogenesis. Probiotics that express enzymes such as NADPH cytochrome P450 reductase, aldehyde reductase, glutathione-S-transferase or N-acetyltransferase, among others, can deactivate procarcinogens such as benzpyrene, heterocyclic amines, nitrosamines and heterocyclic amines. A recombinant strain of *Saccharomyces cerevisiae* that overexpresses NADPH cytochrome P450 reductase has been produced (Blanquet *et al.*, 2001). This strain has been shown *in vivo* in the intestine to convert *trans*-cinnamic acid to *p*-coumaric acid.

### 1.5.4 Antibody production

Recombinant probiotics can be designed to produce single-chain antibodies that can be processed downstream to generate neutralizing antibodies against microbial pathogens, toxins and inflammatory cytokines. An example of this GM technology has been used to create a recombinant *Lactobacillus zeae* that expresses a surface-bound single chain that recognizes the SA I/II adhesion molecule of *Streptococcus mutans* (Lehner *et al.*, 1985). *In vivo* studies in which rats were orally inoculated with *L. zeae* that expressed the single-chain antibody resulted in a marked decrease in *Strep. mutans* counts in the oral cavity and a concomitant decline in the development of dental caries (Lehner *et al.*, 1985). This type of study verifies *in vitro* studies showing recombinant *L. zeae* with SA I/II surface single antibody as capable of causing coagulation with *Strep. mutans* in suspensions. A similar approach has been used to combat *Candida albicans* a major cause of acute vaginitis. Two strains of *Strep. gordonii* have been produced to express and secrete and surface bind a single-chain antibody that exhibits candidacidal activity over a wide concentration range (Beninati *et al.*, 2000). Both *Strep. gordonii* strains colonize the vagina and cleared a *C. albicans* infection in rats. The single-chain secretor strain of *Strep. gordonii* showed a faster reduction of the pathogenic load of *C. albicans*. This type of technology can be used in the future to deactivate toxins and cytokines such as tumor necrosis factor (TNF)-α.

### 1.5.5 Treatment for enzyme deficiencies

Probiotics can be used to produce enzymes that are lacking or abnormally low in humans or animals. *Lactococcus lactis* expressing a lipase gene from *Staphyloccocus hyicus* has been constructed (Drouault *et al.*, 2002). The *L. lactis* could potentially be used to treat pancreatic insufficiency. The *L. lactis* strain requires a nisin promoter. Upon induction it was shown that lipase accumulated to account for up to 15% of total protein intracellularly. Pigs that had their pancreatic duct ligated and then treated with the recombinant *L. lactis* strain had 10% higher fat absorption than untreated controls (Drouault *et al.*, 2002). This type of enzyme replacement technology can be used in the
future to treat a wide variety of enzyme deficiencies resulting from disease, surgical procedures or genetic conditions.

As stated in section 1.5.1, it is desirable to engineer recombinant probiotics with a biocontainment factor. Removing the thymidylate synthetase gene concomitant with adding a gene that expresses a desired product is one example. Another example is to remove the gene that converts L-alanine to D-alanine. Since D-alanine is an essential component of most bacterial cell walls, the ability to inhibit the conversion of L-alanine to D-alanine would limit the growth of the bacteria in the environment. There are numerous other strategies that can be used for biocontainment of probiotics and some of these are discussed in an article on genetically engineered probiotics (Steidler, 2003).

Box 1.1 shows some of the future developments for selecting probiotics for medical applications.

**Box 1.1 Selected future probiotic medical applications**

**Production of anti-inflammatory agents**
Genetically modified to produce IL-10, IL-4, IL-12
Genetically modified to produce curcumin and flavonoids and their analogues

**Detoxification activity**
Toxin sequestration
- Natural selection of strains that bind mycotoxins
- GM strains constructed to bind *E. coli* Shiga toxin or *Shigella dysenteriae* Shiga toxin

Carcinogen detoxification: deactivation via enzymatic expression
- NADPH cytochrome P450 reductase
- Aldehyde reductase
- Glutathione-S-transferase
- N-Acetyltransferase

**Antibody production by GM probiotics**
*Streptococcus mutans*
*Candida albicans*
Other pathogens

**Treatment for enzymatic deficiencies**
Lipase expression
Enzyme deficiencies resulting from surgical procedures, diseases, genetic disorders

**Treatment for hormone deficiencies**
Nasal introduction of GM probiotics expressing for insulin or proinsulin
GM probiotics producing promoter-controlled growth hormone or thyroid hormone

**Natural selection of new probiotic genera**
Propionibacteria
Other genera that are non-pathogenic
1.5.6 Other potential future directions for probiotics for medical use

The use of new genera of microorganisms for probiotic purposes is an additional future direction that has to be considered. The propionibacteria are one such example and their attributes have been discussed in a review article (Ouwehand, 2004). In summary, some of the propionibacteria produce antimicrobial substances such as propionic acid and bacteriocins. In addition, propionibacteria have been shown to have antiviral activity and the ability to adhere to intestinal surfaces. Propionibacteria are capable of stimulating the growth of Bifidobacterium, which is an established probiotic and therefore makes propionibacteria a candidate component of probiotic mixtures. The number of different genera of bacteria, yeast, molds and fungi is large and many of these different organisms have not been tested for beneficial health effects, an area that will be subject to future research.

1.6 CONCLUSIONS

This chapter has outlined the early history of bacterial use of probiotics for the benefit of humankind and the current medical uses and the evidence supporting these health applications. The chapter has also attempted to predict future developments of probiotics based on the latest technological advances in the field of microbial genetics. These future developments will provide new applications for probiotics and an important place for them in the armamentarium against the multiple diseases and disorders that afflict humankind.

REFERENCES


