CHAPTER 1

Understanding the Mechanisms by Which Probiotics Inhibit Gastrointestinal Pathogens

Sinead C. Corr,* **Colin Hill,**† and **Cormac G. M. Gahan**‡

Contents

Abstract

In recent years, there has been a growing interest in the use of probiotic bacteria for the maintenance of general gastrointestinal health and the prevention or treatment of intestinal infections. Whilst probiotics are documented to reduce or prevent specific infectious diseases of the GI tract, the mechanistic basis of this effect remains unclear. It is likely that diverse modes-of-action contribute to inhibition of pathogens in the gut environment and proposed mechanisms include (i) direct antimicrobial activity through production of bacteriocins or inhibitors of virulence gene

* Department of Biochemistry and Immunology, Trinity College Dublin, Ireland
† Department of Microbiology, University College Cork, Ireland
‡ Department of Microbiology, School of Pharmacy and Alimentary Pharmabiotic Centre, University College Cork, Ireland

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expression; (ii) competitive exclusion by competition for binding sites or stimulation of epithelial barrier function; (iii) stimulation of immune responses via increases of sIgA and anti-inflammatory cytokines and regulation of proinflammatory cytokines; and (iv) inhibition of virulence gene or protein expression in gastrointestinal pathogens. In this review, we discuss the modes of action by which probiotic bacteria may reduce gastrointestinal infections, and highlight some recent research which demonstrates the mechanistic basis of probiotic cause and effect.

I. INTRODUCTION

The gastrointestinal tract is a complex ecosystem which can be a reservoir of both beneficial and harmful bacteria. Recently, there has been interest in the role of the gut microbiota in health, and also in the deliberate use of bacterial supplements to influence this microbial community in a manner which could potentially assist in maintaining health and in disease prevention (Holzapfel *et al.*, 1998; Senok *et al.*, 2005). Probiotics (defined as any live microorganism, that when administered to human or animal hosts, has health-promoting benefits) could potentially offer an alternative to conventional therapies such as antibiotics for the prophylaxis or treatment of intestinal infections (Bourlioux *et al.*, 2003; Rolfe, 2000). From various *in vitro* and *in vivo* studies to date, it is clear that probiotics offer great potential in prevention and treatment of infections (Table 1.1). However, a thorough understanding of their mechanisms of action is required to ensure their efficient use. Probiotics are presumed to modulate the indigenous intestinal flora and improve health via a plethora of potential mechanisms of action, such as immunomodulation, direct antagonism, or competitive exclusion (summarized in Fig. 1.1) (Gotteland *et al.*, 2006; Sartor, 2004; Venturi *et al.*, 1999). Probiotics can inhibit growth of enteric pathogens by decreasing luminal pH, the secretion of bactericidal peptides/proteins, or the stimulation of defensin production by epithelial cells (Toure *et al.*, 2003; Zhu *et al.*, 2000). Probiotics can also block attachment to or invasion of the intestinal epithelium by pathogens through blocking of epithelial surface receptors or induction of mucins, large carbohydrate molecules which form a barrier along the epithelial monolayer (Mack *et al.*, 1999; Mattar *et al.*, 2002).

A number of *in vivo* studies have been performed which have determined the probiotic capabilities of such strains. While these studies have been important in demonstrating probiotic efficacy against various infectious diseases, few have specifically identified the mechanistic basis behind the observed benefits, and many rely on *in vitro* data to decipher the possible mechanism of action. However, what has emerged to date is that

TABLE 1.1 A list of potential probiotic strains and their observed beneficial effects

Organism	Effect	Mechanism	Reference
E. coli strain Nissle 1917	Improves epithelial barrier function	Increases tight junction protein expression, ZO-2	Zyrek *et al.* (2007)
L. rhamnosus R0011	Improves epithelial barrier function	Prevents the pathogen-induced drop in transepithelial resistance	Sherman *et al.* (2005)
L. plantarum 299v	Improves epithelial barrier function	Increases extracellular secretion of mucin, MUC3	Mack *et al.* (2003)
VSL#3 probiotic mixture	Improves epithelial barrier function	Induces human β-defensin, hBD-2 gene expression	Schlee *et al.* (2008)
B. breve Yakult	Secretion of inhibitory substances	Production of acetic acid and thus lowering of pH	Asahara *et al.* (2004)
L. johnsonii NCC533	Secretion of inhibitory substances	Production of hydrogen peroxide	Pridmore *et al.* (2008)
L. salivarius UCC118	Secretion of inhibitory substances	Production of bacteriocin	Corr *et al.* (2007b)
L. casei NCDO1205	Immunomodulation	Decrease IL-8 and increase IL-10 response	Corr *et al.* (2007a)
L. rhamnosus GG	Immunomodulation	Activates NF-κB and regulates inflammatory response in macrophages	Miettinen *et al.* (2000)
B. lactis Bb-12	Immunomodulation	Stimulates sIgA	Fukushima *et al.* (1998)
L. rhamnosus GG	Inhibition of virulence factor expression	Reduces expression of genes encoding shiga toxin	Carey *et al.* (2008)
L. plantarum ITM21B	Inhibition of virulence factor expression	Inhibition of urease activity in *Y. enterocolitica*	Lavermicocca *et al.* (2008)

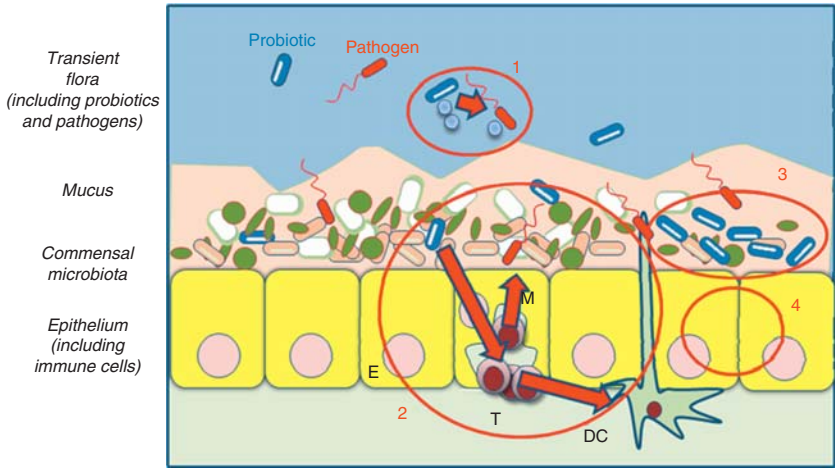

FIGURE 1.1 Probiotics may protect against infection by pathogens through (1) Direct antagonism via bacteriocin production. (2) Immunomodulation via immune cell (T-cell, Dendritic cell) activation. (3) Improvement of epithelial barrier function and competitive exclusion via induction of mucus and blocking of epithelial binding receptors. (4) Strengthening of epithelial tight junctions by increased expression of tight junction proteins, or by a combination of these mechanisms.

the inhibition of pathogens by specific probiotics may represent a highly specific commensal–pathogen interaction. It is clear that further understanding of this phenomenon is required in order to specifically target gastrointestinal pathogens through the use of appropriate probiotic strains.

II. EVIDENCE FOR POTENTIAL MECHANISMS OF ACTION

A. Epithelial barrier function and probiotic signaling

A key mechanism by which probiotics are thought to exert anti-invasive activity is via induction of conformational changes within the epithelial monolayer (Mack *et al.*, 1999). In a recent study of barrier disruption in T84 epithelial cells by infection with enteropathogenic *Escherichia coli*, coincubation with the probiotic *E. coli* strain Nissle 1917 (EcN) or addition of the probiotic after infection abolished this disruption and restored barrier integrity (Zyrek *et al.*, 2007). DNA-microarray analysis identified more than 300 genes exhibiting altered expression following incubation of the epithelial cells with EcN, including expression and distribution of zonula occludens-2 (ZO-2), a tight-junction protein.

Further studies have shown that pretreatment of epithelial monolayers with probiotic bacteria, *Lactobacillus acidophilus* R0052 and *Lactobacillus*

rhamnosus R0011, reduces epithelial injury following exposure to *E. coli* O157:H7 and *E. coli* O127:H6 by preventing the pathogen-induced drop in transepithelial resistance, a measure of barrier integrity (Sherman *et al.*, 2005). These probiotics also reduced the number of foci of rearrangements of α-actinin, indicative of reduced number of attaching and effacing lesions formed in response to *E. coli* O157:H7. In this study, viable lactic acid-producing bacteria were necessary to mediate the observed effects. In another recent study preincubation of Hep-2 cell monolayers with two strains of lactobacilli, *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 and *Lactobacillus plantarum* CIDCA 83114 prior to infection with enterohaemorrhagic *E. coli* (EHEC) minimized F-actin rearrangements and morphological alterations in the cell monolayers (Hugo *et al.*, 2008). These studies collectively indicate that lactobacilli are capable of directly triggering cellular responses in host cells that may impede virulence mechanisms of EHEC.

The exact molecular mechanisms by which probiotics stimulate alterations in epithelial cell function are currently under investigation. Studies have shown that probiotic strains such as the VSL#3 probiotic compound (*Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium breve*, *L. acidophilus*, *Lactobacillus casei*, *L. delbrueckii* subsp. *bulgaricus*, *L. plantarum*, *Streptococcus salivarius* subsp. *thermophilus*) can improve epithelial and mucosal barrier function through production of specific metabolites (Madsen *et al.*, 2001). These include production of short-chain fatty-acids (SCFAs) as by-product of microbial fermentation, such as butyrate which induces epithelial cell differentiation and increases barrier integrity (Cook and Sellin, 1998). *L. acidophilus* has been shown to improve gut barrier function in rats by improving microflora disturbance, increasing occludin expression, and maintaining the gut epithelial tight junction (Qin *et al.*, 2005).

Another physiological change potentially induced by probiotics in the host involves induction or overexpression of mucin. GI tract mucins are large, carbohydrate-rich, high-molecular-weight glycoproteins which are the major components of the mucous layer overlying the intestinal epithelium (Mattar *et al.*, 2002). Mucin forms a physicochemical barrier which protects epithelial cells from chemical, enzymatic, mechanical, and microbial damage, and limits microbial adherence and subsequent invasion (Mack *et al.*, 2003). At least 12 mucin genes have been identified, and of these MUC2 and MUC3 are the predominant ileocolonic mucins (Mack *et al.*, 2003). The MUC2 gene is expressed in goblet cells of the small and large intestine and is the major secreted mucin of the colon (Mack *et al.*, 1999). The membrane-associated mucin MUC3 is not highly expressed in the colon but is expressed on both goblet cells and enterocytes of the small intestine (Chang *et al.*, 1994). Adherence of selected *Lactobacillus* strains (*L. plantarum* 299v, *L. rhamnosus* GG) to the human intestinal HT29 epithelial-cell line induces up-regulation of mucin gene expression, and correlates with increased extracellular secretion of MUC3

(Mack *et al.*, 2003). *L. plantarum* 299v and *L. rhamnosus* GG inhibit the adherence of enteropathogenic *E. coli* to HT29 intestinal epithelial cells via induction or overexpression of mucin (Mack *et al.*, 1999). In an *in vitro* Caco-2 cell model, *L. casei* LGG up-regulates MUC2 expression and has an inhibitory effect on bacterial translocation of the intestinal epithelium (Mattar *et al.*, 2002). Thus, increased expression of intestinal mucin in response to lactobacilli mediates inhibition of adherence of pathogens to intestinal cells. However, analysis of this phenomenon using *in vivo* infection models has not yet been implemented.

Interestingly, Collado and colleagues (2008) have recently shown that specific probiotic strains have the capacity to prevent adhesion of the opportunistic pathogen *Enterobacter sakazakii* to immobilized human mucous *in vitro*. These studies indicate that in addition to inducing upregulation of mucous secretion by the epithelia, specific probiotic strains also have the capacity to competitively exclude or displace pathogens from human mucous as a mechanism for preventing the transient colonization of gastrointestinal pathogens.

Potential probiotic strains can also induce the release of defensins from epithelial cells. These small peptides/proteins are active against bacteria, fungi, and viruses and also stabilize gut barrier function (Furrie *et al.*, 2005). It has been shown that *E. coli* Nissle 1917 induces human β-defensin-2 (hBD-2) gene expression in Caco-2 intestinal epithelial cells (Wehkamp *et al.*, 2004). This induction was mediated by NF-κB and AP-1 signaling pathways. Recently, several strains including *E. coli* Nissle 1917, *L. acidophilus*, *Lactobacillus fermentum*, *Lactobacillus paracasei* subsp. *paracasei*, *Pediococcus pentosaceus*, and the VSL#3 probiotic mixture were found to induce hBD-2 gene expression in Caco-2 cells (Schlee *et al.*, 2008). This was also dependent on mitogen-activated protein kinase (MAPK), NF-κB, and AP-1 signaling pathways (Schlee *et al.*, 2008). This induction of hBD-2 may also enhance mucosal barrier function.

The adhesion ability of some probiotic strains affords probiotic bacteria the capacity to compete with pathogenic bacteria for receptors expressed on epithelial cells, thus blocking contact between epithelial cells and pathogenic bacteria (Sherman *et al.*, 2005; Tsai *et al.*, 2005). In a recent study, BALB/c mice were fed *L. acidophilus* LAP5 or *L. fermentum* LF33 originally isolated from swine and poultry for seven consecutive days before oral challenge with *Salmonella enterica* serovar Typhimurium (Tsai *et al.*, 2005). Numbers of *Salmonella* invading livers and spleens of probiotic-fed mice were significantly lower than placebo controls, and it was thought that the adhesiveness of *Lactobacillus* cells to mouse intestinal epithelium may be an important factor for their antagonistic activity against *Salmonella* invasion *in vivo*. However, this inference was based upon *in vitro* assessment of adherence to intestinal cell lines and was not proven *in vivo* (Tsai *et al.*, 2005).

B. Production of acid and secretion of inhibitory substances

Lactobacillus and *Bifidobacterium* spp. are capable of producing organic acids as end products of metabolism. Selected *Bifidobacterium* species, including *B. breve* strain Yakult, display anti-infectious activity against Shiga toxin-producing *E. coli* (STEC) O157:H7 in mice (Asahara *et al.*, 2004). In this study, *B. breve* Yakult was administered to mice daily for three consecutive days and mice were infected with STEC on day 3. A dramatic decrease in bodyweight and subsequent death was observed in placebo-fed mice, while bodyweight was maintained and no fatalities were observed in *B. breve*-fed mice. This anti-infective activity was thought to be due to production of acetic acid by *B. breve* and lowering of intestinal pH, which had the combined effect of inhibiting Shiga-like toxin (Stx) production (Asahara *et al.*, 2004).

Lactobacillus and *Bifidobacterium* spp. have been shown to impede infection of human intestinal cells by enterohemorrhagic *E. coli* O157:H7 by the combined action of lactic acid and proteinaceous substances (Gopal *et al.*, 2001). An *in vitro* study of the ability of *L. rhamnosus* DR20 and *Bifidobacterium lactis* DR10 to impede infection of differentiated human intestinal cell-lines by *E. coli* O157:H7 found that pretreatment of *E. coli* with concentrated cell-free culture supernatants from these probiotic bacteria significantly reduced numbers of culturable *E. coli* and the invasiveness of this strain (Gopal *et al.*, 2001). The probiotic *E. coli* strain Nissle 1917 interferes with *S.* Typhimurium invasion of human embryonic intestinal epithelial INT407 cells via secretion of inhibitory substances, as shown when the probiotic was separated from the bacteria by a nonpermeable membrane (Altenhoefer *et al.*, 2004). In a previous study, we utilized a similar transwell chamber system to demonstrate that lactobacilli and bifidobacteria (*L. casei*, *L. acidophilus*, *Lactobacillus salivarius*, *B. breve*, *B.* infantis, *B. longum*) are capable of inhibiting *Listeria monocytogenes* invasion of C2Bbe1 epithelial cells in the absence of direct contact through secretion of proteinaceous molecule(s), active at low pH in the case of the lactobacilli strains tested (Corr *et al.*, 2007a). However, the nature of the proteinaceous agent needs to be identified.

Recently, Pridmore and co-workers (2008) have examined the production of hydrogen peroxide by the human gastrointestinal isolate *Lactobacillus johnsonii* NCC533. Through *in silico* analysis of the genome of this potential probiotic strain they identified the means by which hydrogen peroxide is synthesized. Furthermore, they demonstrated that the strain actively produced hydrogen peroxide *in vitro* at levels that were inhibitory for *S.* Typhimurium.

Bacteriocins are compounds with potential anti-microbial activity synthesized by many bacterial species, including lactic acid bacteria (Cotter *et al.*, 2005; Gotteland *et al.*, 2006). As the ability of bacteriocins to

inhibit or kill pathogens is well documented, these molecules represent obvious candidates as mediators of an antipathogen effect. Indeed, bacteriocins have been shown to be necessary *in vivo* for long-term oral colonization by a noncariogenic variant of *Streptococcus mutans* in a therapeutic approach known as replacement therapy (Smith *et al.*, 2006). In a recent study, we demonstrated the ability of *L. salivarius* UCC118 to inhibit *L. monocytogenes* infection of mice, and directly linked this inhibitory effect to production of bacteriocin by *L. salivarius* (Corr *et al.*, 2007b). We showed that mice orally inoculated with *L. salivarius* UCC118 were protected from subsequent oral infection by *L. monocytogenes*. However, a stable mutant of *L. salivarius* UCC118 that is unable to produce the bacteriocin, Abp118, failed to protect mice confirming that bacteriocin production is the primary mediator of protection against this organism. Furthermore, *L. salivarius* UCC118 did not offer any protection when mice were infected with a strain of *L. monocytogenes* expressing the cognate Abp118 bacteriocin immunity protein AbpIM again confirming that the observed protective effect was the result of direct antagonism between *L. salivarius* and the pathogen, mediated by the bacteriocin Abp118.

C. Immunomodulation

Probiotic bacteria are capable of tempering the host inflammatory response to infection and are considered to be important mediators of immune-regulation in the gastrointestinal environment (Corr *et al.*, 2007a; O'Hara *et al.*, 2006). It is likely that this immunomodulatory role is an important factor governing the immune clearance of gastrointestinal pathogens and in preventing the establishment of postinfectious inflammatory conditions (including irritable bowel syndrome, IBS) in the GI tract. Furthermore, chronic inflammatory diseases of the GI tract (including Crohn's disease) are postulated to be linked to underlying infections (by *Mycobacterium avium* subsp. *paratuberculosis* or specific *E. coli* strains) (Darfefeuille-Michaud *et al.*, 2004; Sechi *et al.*, 2004). Probiotic treatment raises the possibility that such chronic infections may be amenable to noninvasive intervention in order to limit the cause of the underlying inflammation.

Probiotic bacteria regulate mucosal immune responses through induction of anti-inflammatory cytokines such as IL-10 and TGF-β, while decreasing expression of proinflammatory cytokines, such as TNF and IFN-γ (Corr *et al.*, 2007a; Di Giacinto *et al.*, 2005; Silva *et al.*, 2004). *B. breve* and *Streptococcus thermophilus* secrete metabolites which inhibit LPS-induced TNF-α secretion from peripheral blood mononuclear cell (PBMC) monolayers (Menard *et al.*, 2004). We demonstrated a significant reduction in interleukin-8 (IL-8) and an increase in IL-10 cytokines secreted from epithelial cells following pretreatment with probiotics

prior to infection with *L. monocytogenes* (Corr *et al.*, 2007a). A number of commensal strains including *L. casei* NCDO1205, *L. salivarius* UCC118, and *B. breve* UCC2003 were capable of inducing this response. Similarly, both *B. infantis 35624* and *L. salivarius UCC118* are capable of reducing *S. typhimurium*-induced proinflammatory responses *in vitro* (O'Hara *et al.*, 2006). These probiotic commensal strains were capable of blunting IL-8 responses and increasing the IL-10 response in an *in vitro* model of *Salmonella* infection.

The mechanistic basis of such responses has been examined by Kelly and co-workers (2004). *Bacteroides thetaiotaomicron* reduces inflammation due to *Salmonella*–TLR5 interactions (Kelly *et al.*, 2004). The mechanism underpinning this anti-inflammatory response was dependent upon PPAR-γ (peroxisome proliferator activated receptor- γ)-mediated inhibition of NF-κB and was directly induced by *B. thetaiotaomicron.* Furthermore, *L. rhamnosus* GG is capable of activating NF-κB and STATs, latent cytoplasmic transcription factors which regulate transcription of genes encoding proteins involved in cytokine signaling and inflammatory responses in macrophages (Miettinen *et al.*, 2000).

Some probiotics also stimulate secretory IgA production and activate regulatory T cells (Fukushima *et al.*, 1998). These effects have been seen in human studies and demonstrate that anti-Polio sIgA is increased in those administered a probiotic preparation viable *B. lactis* Bb-12. Similarly, an increase in IgA^+ cells was witnessed in mice administered *L. casei* (Galdeano and Perdigón, 2006). However, other studies have demonstrated that stimulation of sIgA in humans is stimulated by a prebiotic preparation but not by administration of live probiotic (*Bifidobacterium animalis*) (Bakker-Zierikzee *et al.*, 2006).

Inflammatory conditions of the GI tract may be initiated by a disregulated local immune response to the normal microbiota and are host dependent (Sartor, 2003; Shanahan, 2001). However, a subset of IBS patients experience symptoms following gastrointestinal infection (post-infectious IBS). In addition, underlying infection has been proposed as a possible trigger in Crohn's disease and both *M. avium* subsp. *paratuberculosis* or adherent invasive *E. coli* (AIEC) have been suggested as possible sources of inflammation (Darfefeuille-Michaud *et al.*, 2004; Sechi *et al.*, 2004). Ingrassia and co-workers have demonstrated that *L. casei* DN-114 001 is capable of inhibiting AIEC strains isolated from Crohn's disease patients in cell culture models of infection, suggesting that probiotic intervention may present a future strategy for limiting the pathogenesis of a potential trigger of inflammation in Crohn's disease (Ingrassia *et al.*, 2005).

Indeed, human studies indicate that specific probiotic strains can reduce symptoms of IBS through immunomodulation (Kajander *et al.*, 2008; O'Mahony *et al.*, 2001) and may have promise for the treatment of

inflammatory bowel disease (IBD) although further research is needed (Hedin *et al.*, 2007). Recently, *L. acidophilus* has been shown to reduce the inflammatory response in gastric epithelial cells via production of conjugated linoleic acids (CLA) (Kim *et al.*, 2008). In this study, conditioned medium containing *L. acidophilus*-producing CLA interacts with IκB kinase inducing phosphorylation of inhibitory IκBα leading to its dissociation from NF-κB and thus, NF-κB activation. *Lactobacillus reuteri* has recently been shown to secrete factors which potentiate apoptosis by stabilizing IκBα degradation and inhibiting nuclear translocation of p65, thus leading to suppression of NF-κB-dependent gene products that mediate cell proliferation and cell survival including Cox-2 and Bcl-2, respectively (Iyer *et al.*, 2008). Promotion of cell apoptosis serves as a therapy to prevent colorectal cancer and IBD (Iyer *et al.*, 2008).

The VSL#3 probiotic mix which contains viable lyophilized bifidobacteria (*B. longum*, *B. infantis*, and *B. breve*), lactobacilli (*L. acidophilus*, *L. casei*, *L. delbrueckii* subsp. *bulgaricus*, and *L. plantarum*), and *S. salivarius* subsp. *thermophilus* (VSL Pharmaceuticals, Fort Lauderdale, FL), can significantly modulate the immune response and has been shown to play a role in maintenance of treatment in ulcerative colitis (Venturi *et al.*, 1999). In this study, patients with ulcerative colitis in remission were given VSL#3 for 12 months and it was shown that of those taking the probiotic, the majority remained in remission throughout the study period. Recently, it was shown that culturing human blood dendritic cells with cell-wall components of the probiotic mixture VSL#3 induced dendritic cell maturation and up-regulated production of IL-10 (Hart *et al.*, 2004). Dendritic cells, which play an important role in early bacterial recognition and in T-cell responses, may be central mediators of these probiotic effects. Indeed, administration of VSL#3 is associated with an early increase in IL-10 production and regulatory $CD4^+$ T cells bearing surface TGF-β in murine models of colitis, while human studies have shown increased mucosal regulatory T cells and a reduction in pouchitis disease activity (Di Giacinto *et al.*, 2005; Pronio *et al.*, 2008). *L. acidophilus* strain L-92 has recently been shown to regulate both Th1 and Th2 cytokine responses in BALB/c mice possibly through modulation of TGF-β-associated activation of T-regulatory cells, suggesting a potential therapy for Th1- and Th2-mediated disease including autoimmune disease and inflammatory diseases (Torii *et al.*, 2007).

D. Inhibition of virulence factor expression

A potential mechanism of action by which potential probiotic strains may impede pathogens is through the modulation of gene and/or protein expression patterns through bacterial signaling mechanisms. Interestingly, cell-free supernatants of *L. acidophilus* have been shown to inhibit

quorum sensing and virulence gene expression in *E. coli* O157:H7 but did not affect expression of shiga toxin in this strain (Medellin-Peña *et al.*, 2007). Other researchers have utilized microarray analyses to investigate the global transcriptional changes in *E. coli* O157:H7 following coincubation with *L. rhamnosus GG* (LGG). Results indicated that LGG coincubation reduces expression of the *stx* genes encoding shiga toxin production in *E. coli* O157:H7 (Carey *et al.*, 2008). Subsequently, a variety of *Lactobacillus*, *Pediococcus*, and *Bifidobacterium* strains (*L. rhamnosus GG, Lactobacillus curvatus, L. plantarum, Lactobacillus jensenii, L. acidophilus, L. casei, L. reuteri, Pediococcus acidilactici, Pediococcus cerevisiae, P. pentosaceus, Bifidobacterium thermophilum, Bifidobacterium boum, Bifidobacterium suis, and B. animalis*) were shown to repress *stxA* expression in this model system, suggesting a global mechanism by which the microbiota could impede virulence factor expression in this pathogen (Carey *et al.*, 2008). Similarly, a recent study examined the ability of a variety of potential probiotic strains to inhibit the ureolytic pathogen *Yersinia enterocolitica* (Lavermicocca *et al.*, 2008). They determined that one probiotic strain, *L. plantarum* ITM21B, was capable of inhibiting urease activity in the pathogen. Overall, it is likely that future studies will uncover the regulatory networks that govern signaling mechanisms between pathogens and commensals.

III. CONCLUSIONS

There is mounting evidence to support a role for probiotics as an alternative to conventional methods for prevention and treatment of intestinal diseases and inflammatory disorders. The introduction of probiotic organisms has been proposed to improve digestive function (Savaiano *et al.*, 1984), reduce chronic inflammation (Di Giacinto *et al.*, 2005; O'Hara *et al.*, 2006), and improve recovery from foodborne disease (Aiba *et al.*, 1998). Previous work using rodent models of disease has demonstrated a role for probiotics in the amelioration of infections caused by *Helicobacter pylori* (Gotteland *et al.*, 2006), *Citrobacter rodentium* (a murine model of Enteropathogenic *E. coli* (EPEC)) (Johnson-Henry *et al.*, 2005) and *S.* Typhimurium (Silva *et al.*, 2004) and clinical trials have shown that administration of probiotics can significantly improve eradication of *H. pylori* in infected patients (Gotteland *et al.*, 2006). *In vitro* analyses have indicated that regulation of mucous production by probiotics can prevent colonization by EPEC (Mack *et al.*, 1999) and there is an apparent correlation between immunomodulation by probiotics and elimination of foodborne pathogens (Jijon *et al.*, 2004). Efficient use of probiotic therapies will require that the precise mechanism(s) by which specific probiotic strains exert their effect is identified. While the molecular details underpinning probiotic modes of action remain almost entirely unknown,

recently there has been significant progress towards understanding how probiotics exert their beneficial effects at the molecular level. This suggests that the next phase of therapeutic development will represent a ''bugs to drugs'' approach whereby probiotic-based therapeutic agents are developed as specific pharmabiotics (O'Hara and Shanahan, 2007).

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