

# Probiotics: An Emerging Therapy

G.C. O'Sullivan\*, P. Kelly, S. O'Halloran, C. Collins, J.K. Collins, C. Dunne and F. Shanahan

*Cork Cancer Research Centre and Alimentary Pharmabiotic Centre, University College Cork, National University of Ireland, Cork, Ireland*

**Abstract:** There is considerable clinical interest in the utility of probiotic therapy – the feeding of (live) non-pathogenic bacteria, originally derived from the alimentary tract, for disease treatment or health promotion. The microflora of the gastrointestinal tract is essential for mucosal protection, for immune education and for metabolism of fecal residue. Physiological disturbances of these processes, when they occur, result from: i) alteration of a microbial ecosystem, originally conserved by evolution; ii) reduced consumption of microorganisms; iii) invasion of pathogens; or iv) modern interventions. Recent data support the use of proven probiotic organisms in prevention and treatment of flora-related gastrointestinal disorders including inflammatory bowel disease, infectious and antibiotic related diarrheas, and post-resection disorders including pouchitis. Therapeutic activity of probiotic bacteria can be due to competition with pathogens for nutrients and mucosal adherence, production of antimicrobial substances, and modulation of mucosal immune functions. Although a promising treatment, controlled clinical trials are necessary to validate the benefit of probiotics

**Key Words:** Probiotics, prebiotics, synbiotics, enteric microflora, mucosal adherence, inflammatory bowel disease, pouchitis, colon cancer.

## INTRODUCTION

Probiotics have been defined as “live microbial food supplements which beneficially affect the host by improving the intestinal microflora balance” [1], or more broadly as “living microorganisms, which upon ingestion exert health benefits beyond inherent general nutrition” [2]. Originally available as alternative therapies or as health foods, their reputation in the medical community suffered through extravagant claims of benefits relating to ingestion of varying microbial strains, many of which had neither demonstrable probiotic activity nor viability in the gastrointestinal tract. There are, more recently, increasing experimental and clinical data to support the use of proven probiotic organisms in prevention and treatment of many gastrointestinal disorders. These include inflammatory bowel disease, infectious and antibiotic related diarrheas, and post-resection disorders including pouchitis [3-6]. Microbial interference therapy - the use of nonpathogenic microorganisms to eliminate pathogens - has been advocated as an alternative, where possible, to antibiotics in an effort to reduce the growing problem of antibiotic-resistant bacteria [7]. The rationale of treatment is to promote health by altering the ecological balance of the microbial flora through the ingestion of probiotics or dietary compounds which favor growth of probiotic bacteria (prebiotics), or both (synbiotics) [3, 4, 7-9].

## GASTROINTESTINAL FLORA

Co-evolution led to a symbiotic relationship between eukaryotes and prokaryotes with the development of sophisticated bi-directional signaling systems in mucosal epithelia and lymphocytes [9, 10]. It has been clearly

established that the gastrointestinal flora are essential for mucosal protection and immune education. Germ-free animals are delicate, have hypoplastic epithelia, impaired peristalsis and reductions in mucosal immune function and lymphoid tissue mass. Re-introduction of flora to the gnotobiotic animals restores intestinal function, mucosal proliferation, immune development and animal growth [10-13]. This clearly suggests that mucosal defence by the normal flora is determined not only by competition with potential pathogens but also by the exchange of regulatory and trophic signals with the enteric and immune cells of the mucosa.

Within the intestinal lumen, there exists a complex but relatively stable microbial ecosystem containing more bacteria than there are cells in the entire human body, accounting for approximately 1kg of intestinal contents or 75% of the wet weight of faeces. Each gram of faeces contains approximately  $1 \times 10^{12}$  microbes, of an estimated 50 genera, belonging to over 400 separate species [9, 11, 12].

Knowledge of the normal flora is still relatively meagre - most of the microorganisms present have not been characterized and at least half of the microbes are as yet unculturable. Faecal samples are most frequently used to study the microflora, but the microbial inhabitants of faecal specimens represent the luminal environment which differ from the mucosal surface and crypt flora and are indicative more of the distal colon than the proximal intestines [14-17].

Despite their shortcomings, conventional analytical techniques have demonstrated that this complex microbial community differs in composition along the length of the gut with an increasing gradient of indigenous microbes from stomach to colon. There is also considerable variability across the diameter of the gastrointestinal tract, which contains both rapidly transiting and relatively persistent components [16, 17]. More specifically, the anaerobic to facultative ratio is approximately 1,000 : 1 at the center of the lumen and is less

\*Address correspondence to this author at the Cork Cancer Research Centre, Mercy University Hospital, National University of Ireland, Cork, Ireland; Tel: +353-21-4271971; Fax: +353-21-4279896; E-mail: gerald@iol.ie

than 10: 1 at the epithelial surface. Within the intestinal crypts it is likely that the bacteria derive oxygen by intimate mucosal contact, as here the microbial species are enriched for facultative types, which remain relatively constant and persist for several weeks. Also, strict anaerobes do not propagate due to the low oxygen tension in the crypt environment. Thus, the symbiotic relationship between the host and intestinal bacteria is determined by the balanced relationship of two specialized zones of microbial activity; paramucosal bacteria along with the intact mucosa provide a barrier against pathogenic organisms / antigens / noxious agents and a more central metabolically-active zone within the faecal residue dependant on propagation of strict anaerobic microorganisms, which if displaced may have the potential to become pathogenic.

The gastrointestinal microflora has been described as the most adaptable and renewable metabolic organ of the body, the composition and activities of which can affect both intestinal and systemic physiology [15]. Such influence is not surprising as dietary by-products, intestinal secretions and epithelial cells shed into the lumen are potential substrates for microbial transformation. Relative to other regions of the gut, the large intestine contains the most complex and diverse microbial population demonstrating a level of metabolic activity comparable to that of the liver. The principal end products of microbial fermentation of undigested residue in the distal small bowel and proximal colon are (in addition to carbon dioxide, methane and hydrogen) essential mucosal nutrients including amino acids (arginine, cysteine and glutamine) and short chain fatty acids (SCFA: acetate, propionate and butyrate) [18-20]. These SCFA serve as an energy source for the host, providing 10-30% of basal metabolic requirements including energy for liver cells, colonocytes and peripheral tissues with only about 5% excreted in the faeces [18, 19]. This is also an important energy source in patients with malabsorption and short gut syndrome. SCFA influence mucosal growth and blood flow in the colon, promote sodium and water absorption and have been shown to improve inflammatory conditions of gut including ulcerative colitis, diversion colitis and pouchitis [19, 21, 22]. In addition to the beneficial end products of residue metabolism, there are also toxic derivatives of microbial proteolysis (such as ammonia, phenols, indoles and amines) [15, 23]. The levels and influence of these toxic agents vary with the composition of both diet and microbial flora. Besides fermentation, the metabolic repertoire of the flora includes synthesis of vitamins K and B complex, secondary bile acid production, neutralisation of dietary carcinogens such as nitrosamines, and conversion to active metabolites of some prodrugs (e.g., sulphasalazine). There are also direct negative influences of some members of the microflora including production of toxic and even genotoxic compounds by removal of conjugates, previously applied in the liver, by bacterial enzymes including -glucuronidase and -glycosidase [24, 25].

With few exceptions, ingested dietary bacteria are unable to survive the acidic conditions of the stomach, exposure to bile acids and pancreatic enzymes in the duodenum, epithelial mucin secretion and cell turnover [20]. The stomach is subject to low pH and relatively short transit times. Therefore, the levels of microbes recoverable by conventional

techniques are approximately  $10^3$  bacteria/ml of contents, increasing temporarily by 100-1000 fold, with the increase in pH following a meal. Gram-positive species, predominantly lactobacilli, are typical isolates due to their acid-tolerance. *Helicobacter pylori* has adapted to the gastric microclimate colonizing the antrum in approximately 50% of individuals. In the duodenum, the combination of short transit times with the antibacterial properties of biliary and pancreatic secretions represents a hostile environment for transiting microorganisms. Therefore, it is not until the distal small intestine (ileum) that both microbial concentration ( $10^8$  bacteria/ml) and diversity (streptococci, lactobacilli, bifidobacteria, bacteroides, fusobacteria and Enterobacteriaceae) increase significantly. Below the ileo-caecal valve, the bacterial numbers exceed  $10^{10}$  -  $10^{11}$  cells/gram. Of those we can readily study, the strict anaerobes such as clostridia species, *Bacteroides*, *Eubacterium*, *Bifidobacterium* and *Peptostreptococcus* dominate (at levels of approximately  $10^{10}$ - $10^{11}$  cells/g) although Enterobacteriaceae, streptococci and *Lactobacillus* species are also present at concentrations of about  $10^8$  cells/g. In contrast to large inter-individual variations in gut flora, the composition of major bacterial groups within a specific individual remains relatively constant [20].

The indigenous intestinal (autochthonous) microbiota act as a further barrier against any transient (allochthonous) potential pathogens by competing for nutrients and mucosal adherence and by local production of agents, which are active against pathogens. However, a variety of potentially harmful micro-organisms are members of the normal gut microflora. These pathogens may be autochthonous within the intestinal niche, held in check by normal intestinal microbial activities and become pathogenic only when that ecosystem undergoes abnormal changes [13, 17, 26]. Alternatively, particular microbes may be autochthonous within a specific region of the gastrointestinal tract but allochthonous within another. Examples of the effects of pathogen proliferation include: pseudomembranous colitis due to toxin production by *Clostridium difficile* usually associated with antibiotic treatment; post-surgical sepsis by *Escherichia coli*, *Enterococcus faecalis* and *Enterococcus faecium*; and intra-abdominal abscesses due to, amongst others, *Bacteroides fragilis*. Depending on genetic and other host related susceptibility factors the intestinal flora may contribute to the pathogenic processes as exemplified by intestinal overgrowth phenomenon and bacterial translocation in the setting of immune or microvascular compromise, motility disorders and blind loop syndromes. The initiation and maintenance of intestinal disorders, such as ulcerative colitis and Crohn's disease, have been attributed to hyperimmune reactions to luminal products of some bacterial members of the gut flora in genetically susceptible individuals [27].

The processes involved in the acquisition and subsequent establishment of the intestinal microbial communities are complex, involving the succession of microbial populations within specific regions of the gut in addition to microbe-host interactions. During and after natural child delivery, the foetus (previously sterile *in utero*) is subject to microbial contamination from the maternal genital tract area and the surrounding environment (air, nursing staff, neonatal care units, etc.), levels of which are influenced by the duration of

the delivery process. In contrast, the initial microbial contact of new-born infants following caesarean delivery originates from air, nursing staff and neonatal care environments [28, 29].

Following delivery (natural or caesarean), neonates are continually exposed to food-borne micro-organisms, both beneficial and otherwise. Healthy breast milk, for instance, contains significant numbers of bacteria. These commensal bacteria include, streptococci, lactobacilli, corynebacteria, micrococci, propionibacteria and, most significantly, *Bifidobacterium* species. For infants who are breast-fed, the culturable microbial composition of their faecal flora is dominated by *Bifidobacterium* species with concomitantly decreased levels of *E. coli*, streptococci, bacteroides and *Clostridium* species. In contrast, formula-fed babies have a more complex form with equal dominance between bifidobacteria and potentially pathogenic anaerobes [28, 29]. This has recently influenced the development of infant feed formulas with bifidogenic properties similar to human breast milk – in an effort to reduce the risk of development of enterocolitis. On introduction of supplementary solids and weaning, the microbial profile of the breast-fed resembles that of the formula-fed infant and bifidobacteria no longer dominate the faecal flora. By 12-24 months after birth, irrespective of the feeding regime, the infant flora has become considerably more complex and resembles that of the adult.

## MODULATION OF HOST IMMUNE RESPONSES

The complex gastrointestinal microbial load is required for the normal development and homeostasis of the humoral and cellular mucosal immune systems. It is the interactions between the mucosal immune systems and the enteric microflora which maintain the physiologically normal state of inflammation or activation of gut-associated lymphoid tissue (G.A.L.T: Peyer's patches, lymphoid follicles, lymphocytes and mesenteric lymph node cells) throughout life [12, 26, 30-32]. GALT is the largest immune organ in the human body and lumenal antigens presented at these sites can result in the stimulation of appropriate T and B cells, the establishment of cytokine networks, and the secretion of antibodies into the gastrointestinal tract. The gastrointestinal mucosa is the largest surface at which the host interacts with the external environment. Besides effector systems, specific control or tolerance mechanisms are in place to regulate immune responsiveness to potentially antigenic food fragments and to distinguish non-pathogenic adherent bacteria from invasive pathogens which have the potential to cause significant damage to the host.

In the absence of an enteric flora, the intestinal immune system is underdeveloped, as demonstrated in germ free animal models, and different aspects of systemic immune function are diminished. These include impaired immune effector components (e.g., macrophage phagocytic ability and immunoglobulin production) and impaired toleragenic components due to failure of oral tolerance development [10-12, 26, 27, 30-32].

Modern lifestyle changes impose stresses on systems genetically adapted over millions of years. The consumption of food containing microorganisms has dramatically

reduced, and as a consequence, the developing mucosal immune system sees a different microflora and fewer pathogens than paleolithic man [33]. The increases in observed incidence and severity of allergies and conditions such as inflammatory bowel disease in the Western world has been linked with increases in standards of hygiene and sanitation, concomitant with a decrease in the number and range of infectious challenges encountered by the growing and developing host. This lack of immune education distorts the development of the immune system and may allow the host to over-react to non-pathogenic antigen-containing commensal flora, resulting in inflammatory damage and/or allergy and autoimmunity [27, 34].

## PROBIOTIC BACTERIA

Bacteria associated with probiotic activity are most commonly lactobacilli and bifidobacteria but other non pathogenic organisms such as certain strains of *E. coli* and non-bacterial organisms such as *Saccharomyces boulardii* have been used [2]. Most of these organisms, originally derived from faeces of healthy individuals, are safe for human consumption and are available over the counter. Because of continued skepticism of such products European Union-funded research groups including medical, scientific and industrial interests agreed criteria for selection and assessment of probiotics.

To fulfill these criteria probiotic micro-organisms should be of human origin, demonstrate non-pathogenic behaviour, even in immuno-compromised hosts, exhibit resistance to technological processes, prove resistant to gastric acid and bile, adhere to gut epithelial tissue, and be able to persist, albeit for short periods, in the gastrointestinal tract, produce antimicrobial substances, modulate immune responses, and have the ability to influence metabolic activities (e.g., cholesterol assimilation, lactase activity, vitamin production).

## ISOLATION OF POTENTIAL PROBIOTIC BACTERIA

In the development of probiotic foods intended for human consumption, strains of lactobacilli and bifidobacteria have been most commonly used (see Table 1). This is primarily due to the perception that they are desirable members of the intestinal microflora. In addition, these bacteria have traditionally been used in the production of fermented dairy products and have "GRAS: generally regarded as safe" status [3, 35].

## INDICATIONS FOR PROBIOTICS

### Infection Related Gastrointestinal Disorders

Worldwide, acute diarrhea affects 3-5 billion patients per annum and is responsible for 3-5 million deaths. Post infective problems of increasing concern include asymptomatic carriage of pathogens, emergence of resistant variants, longterm intestinal dysfunction and incurability in the presence of immune compromise and structural anomalies of the gut. A role for probiotics in defence against enteric pathogens, in treatment of contaminated bowel syndromes or in reconstituting the intestinal flora after antibiotic usage, is conceptually sound. Probiotic lactobacilli have shown efficacy in treating infection-based diarrheas due to either

**Table 1. Successful probiotic Bacteria and their Reported Effects**

Strain	Reported effects
<i>Lactobacillus acidophilus</i> LA1	Immune enhancer, adheres to human intestinal cells, modifies intestinal microflora
<i>Lactobacillus acidophilus</i> NCFB 1748	Decreases fecal mutagenicity, prevents radiotherapy related diarrhea, used in treatment of constipation
<i>Lactobacillus</i> GG (ATCC 53013)	Prevents diarrhoea associated with antibiotics, rotavirus and <i>Cl. Difficile</i> . Used in the treatment of Crohn's disease,
<i>Lactobacillus casei</i> Shirota	Treatment of rotavirus diarrhoea, modifies intestinal microflora, has positive effects in the treatment of superficial bladder cancer, immune enhancer.
<i>Lactobacillus bulgarius</i>	Treatment of rotavirus and viral diarrhoea, modifies intestinal microflora.
<i>Bifidobacterium bifidum</i>	Treatment of rotavirus and viral diarrhoeas modifies intestinal microflora.
<i>Lactobacillus gasseri</i> (ADH)	survives in the gastrointestinal environment.
<i>Lactobacillus reuteri</i>	Colonises the intestinal tract, mainly animal studies, an emerging human probiotic.
<i>Lactobacillus plantarum</i>	Safe in immunosuppressed patients, inhibits bacterial translocation and secondary septic responses
<i>Lactobacillus salivarius</i> UCC118	Reduces faecal coliforms, <i>Cl. perfringens</i> and enterococci levels attenuate gastrointestinal inflammation, prevents tumour formation in an IL-10 knockout mouse model.
<i>Bifidobacterium infantis</i> UCC 35624	Reduces clostridia levels and increases lactobacilli and bifidobacteria, increased blood phagocytic activity, Reduces inflammation in CD45Rbhi mouse model.

[Modified from Salminen, 1996; Holzapfel, 1998]

rotavirus, *Clostridium difficile* or those associated with travel [36-38]. Therapeutic activity is due in part to the ability of the microbes to attach to enterocytes and colonise the crypts [3, 4, 9, 39, 40]. While this may lead to competitive displacement of intestinal pathogens, engagement of cell membrane receptors activates signalling events that lead to nitric oxide production, cytokine synthesis including interferons, and cell resistance to viral attack [41]. Other important modes of activity involve reduction of numbers of pathogens by competitive and bactericidal influences as previously described. In HIV positive children suffering from diarrhea secondary to malabsorption associated with bacterial overgrowth, oral administration of *Lactobacillus plantarum* 229v was found to be safe and to have beneficial effects on symptoms and on the immune responses [42]. Thus probiotic therapy is safe in immunosuppressed patients.

A policy of microbial interference therapy has been suggested for prevention and treatment of antibiotic resistant organisms in the presence of immunosuppression, or for reduction of the translocation of enteric microbes against the background of systemic inflammatory response in critically ill patients [3, 4, 7, 43].

### Inflammatory Bowel Disease

A rationale for treating Crohns disease or ulcerative colitis is emerging [27, 44-48]. A number of observations implicate luminal products from some of the resident flora in driving the mucosal inflammatory response in genetically susceptible individuals [49]. The intestinal mucosa is said to be in a state of controlled inflammation, which is finely balanced by the checking influence of regulatory T cells on effector T cell subsets. When regulation becomes defective the activities of the effector T cells predominate and manifest

as pathological inflammation. Since the normal intestinal flora vary in capacity to drive mucosal inflammation, some bacteria such as lactobacilli and bifidobacteria with apparent anti-inflammatory activity have been selected as candidates for probiotic treatment of inflammatory bowel disease. The results in treating different models of murine enterocolitis are encouraging but highlight variability in probiotic performance between organisms depending on the experimental model [50-52]. Thus, as yet, there is uncertainty whether a particular probiotic strain would be effective in all patients or even in the same patient at different stages in the clinical course of the disease. Two controlled trials of a non-pathogenic strain of *E. coli* in ulcerative colitis have suggested efficacy similar to that of mesalazine [53, 54]. In acute Crohn's disease a preliminary open trial found that *Lactobacillus salivarius* UCC118 was safe and would reduce the requirement for corticosteroids in the majority of patients. European Commission-funded clinical trials assessing well-characterized strains of lactobacilli and bifidobacteria in maintenance of remission of Crohn's and ulcerative colitis are currently in progress.

### Intestinal Contamination Post Surgery

Probiotic therapy has been recommended for prevention and treatment of bacterial overgrowth syndromes after intestinal resection and reconstruction. A hyper-response to products of bacterial overgrowth is thought to be the mechanism of inflammation and diarrhea complicating ileoanal pouch reconstructions in ulcerative colitis patients. This may in part be related to genetic susceptibility and the presence of bacteria peculiar to the ulcerative colitis, as pouchitis is rarely seen in the pouches of patients who were operated on for familial polyposis coli [55-57].

A mixture of probiotic organisms has been successfully used in a controlled trial of maintenance treatment in patients with chronic pouchitis [58, 59]. While combined bacterial preparations are attractive and suggest wider therapeutic advantage, more detailed information on the activity of the individual bacterial components and interstrain competition is necessary.

### Recurrent Urinary Tract Infections

Recurrent urinary tract infection in the absence of anatomical abnormalities is a major problem for many women. The female genital tract is normally colonized by enteric organisms from the gut and when present invasive pathogens gain easy access to the bladder. Probiotic bacteria have been shown to colonise the vagina from the bowel within days of consumption, suggesting that these bacteria would be effective in protection of the female urogenital tract from invasive pathogens [60].

Patients with neurogenic bladders requiring catheterization develop frequent episodes of symptomatic urinary tract infections which are sometimes life threatening. A recent pilot study of 44 patients found that when non-pathogenic *E. coli* were deliberately inoculated into the bladder, the infection rates were reduced 33 fold when the patients were colonized compared with those where colonization was unsuccessful [61]. This strategy extends the application of bacteriotherapy, and could help patients with spinal cord injury or frail elderly people.

### Probiotic Colonization of Babies to Prevent Atopic Diseases

Atopic diseases such as eczema, allergic rhinitis and asthma are chronic disorders of increasing importance in the Western world [62, 63]. In a double blind, randomized, placebo-controlled trial of prevention of atopic diseases in high-risk children *Lactobacillus GG* was administered prenatally to mothers and postnatally for 6 months to their babies. The frequency of atopic eczema in the probiotic treated group was half that of the placebo group [15 of 64 (23%) and 31 of 68 (46%) respectively,  $p=0.008$ ] [64]. While further confirmatory studies are desirable, this study supports the view that risk of allergic diseases may, at least in part, be determined by selective modulatory influences of some members of the gut microflora on the developing immune system. This risk can be reduced by development of immune tolerance systems through exposure to probiotics in infancy – the molecular and cellular basis of which remains to be clarified.

## MORE CIRCUMSPECT INDICATIONS

### Prevention of Colon Cancer

Probiotics may also have a role in prevention of colon cancer and trials are now feasible for “at risk” individuals [27, 65]. Several overlapping mechanisms for probiotic-associated anticancer effects have emerged including a reduction in the ability of the enteric flora to convert dietary procarcinogens to carcinogens [66-68]. Other mechanisms may involve direct modulation of mucosal immune and proliferative functions - in a murine model of enterocolitis,

probiotic therapy was found to reduce the severity of inflammation and the rate of progression from colitis through dysplasia to invasive colon cancer [45, 52].

### Reduction of Blood Lipids

Feeding a diversity of probiotics has been shown to lower blood cholesterol in a number of animal models. A number of mechanisms have been proposed for cholesterol lowering including binding of cholesterol by the bacteria with increased faecal excretion, assimilation of the cholesterol molecule, and deconjugation of bile acids with increased rate of bile acid excretion [69-74]. To date, clinical studies in this area have been inconclusive and more rigorous double blind studies with placebo-fed controls are necessary.

### Enhancing Immune Function

Probiotic consumption has been recommended for immune modulation and general health promotion. The precise mechanisms of immune modulation by probiotics have not been elucidated but they are known to influence both non-specific and specific immune responses in animal models and in humans [75, 76]. Several human studies have confirmed increased resistance to bacteria through augmentation of phagocytic capability of peripheral blood leukocytes coincident with gastrointestinal colonization with probiotic lactobacilli [31, 12]. This is associated with increased production of pro-inflammatory (IL-12, IL-6, TNF- and IFN- ) and anti-inflammatory (IL-10) mediators. The overall influence of these mediators is uncertain and depends on many factors such as cytokine clearances, target cell context and responsiveness. A systemic modulatory role is also suggested by the finding of transitory elevation of IL10 levels in atopic children on feeding probiotics [63].

Some probiotic bacteria influence immunoglobulin production, thus creating a gut immunological barrier, an increase in numbers of IgA and IgM producing cells occurs in the intestinal mucosa with enhanced secretion of secretory IgA (sIgA) and IgM and responsiveness to pathogens and to potentially sensitizing antigens. Neutralisation of allergens and prevention of their absorption is considered important in forestalling hypersensitivity responses.

### Oral Vaccination and Treatment Using Recombinant Probiotic Bacteria

The majority of pathogenic organisms gain entry via mucosal surfaces. Efficient vaccine delivery to these sites may protect against invasion by particular infectious agents. Oral vaccination strategies have concentrated, to date, on the use of attenuated live pathogenic organisms or purified encapsulated antigens. Lactic acid bacteria, modified to deliver antigens derived from infectious agents, may provide an attractive alternative as these bacteria are considered to be safe for human consumption and are currently undergoing European Commission-supported studies [77]. The delivery of allergens by orally administered recombinant lactobacilli was found to inhibit Th1 and Th2 subset responses, suggesting potential of a molecular vaccine approach to allergic disorders [78].

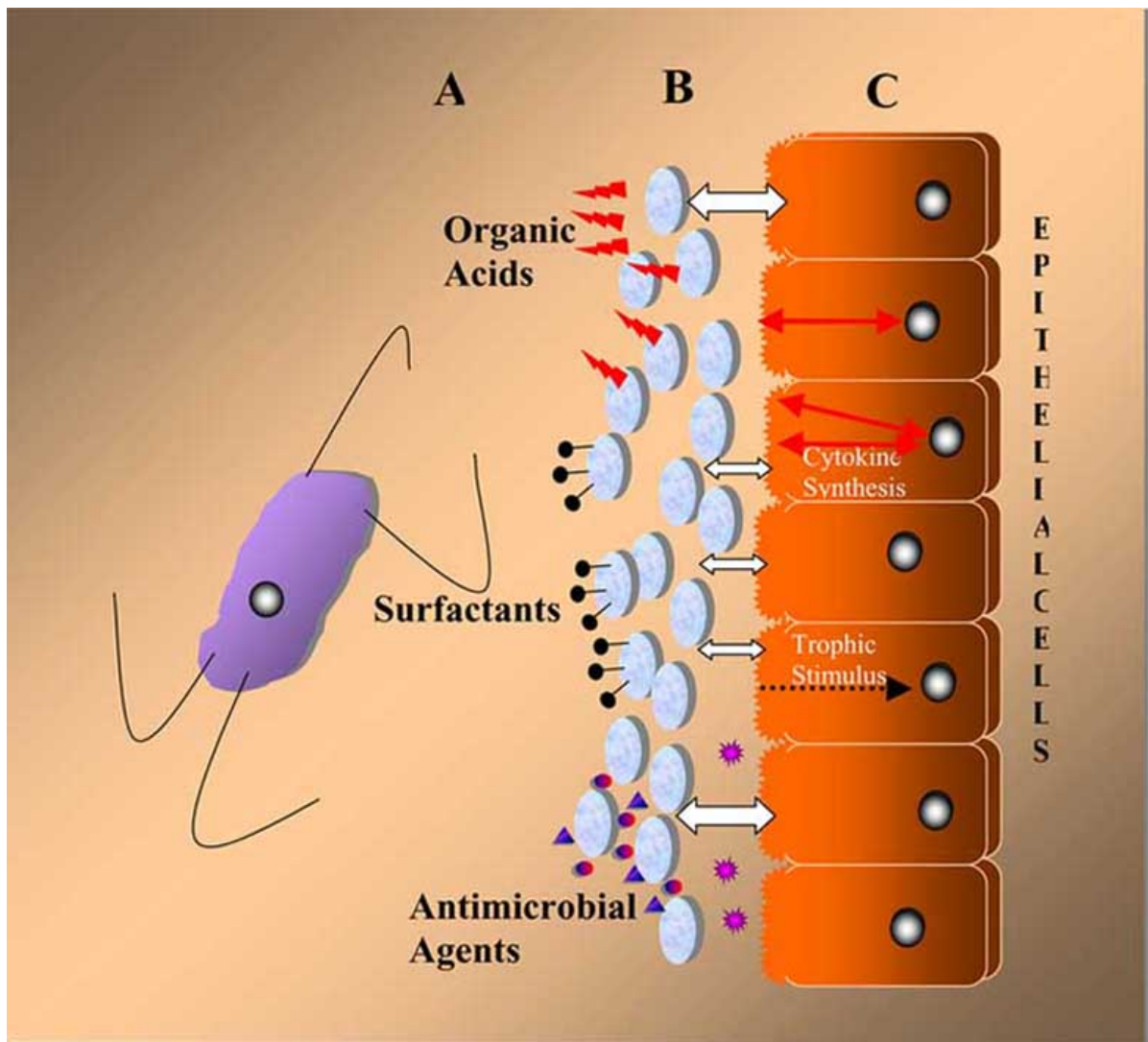


Figure 1. The interface between adhering probiotic bacteria and gut epithelial cells. Probiotic activity of the bacteria is achieved by: **A** secretion of organic acids, surfactants, antimicrobial agents including bacteriocins and hydrogen peroxides. **B**. Bacteria compete with pathogens by adherence and signal exchange through epithelial cell receptors. **C**. Probiotic signals are trophic to the epithelial cell and cytokine synthesis making the cell less vulnerable to attack by pathogens including viruses

**CONCLUDING PERSPECTIVE**

Probiotics are safe for human consumption and their therapeutic role will increase with improved understanding of the microbial flora and its relationship to human physiology and disease pathogenesis. Further organisms will become available which, through either genetic selection or manipulation will possess defined physiological functions that are applicable to health maintenance or to disease prevention and treatment. Newer molecular technologies are being applied to identify the unculturable intestinal bacteria

and to define their role in the luminal ecosystem and in the maintenance of mucosal function. Unravelling the mechanisms that underpin the dependency between the host and the intestinal flora will lead to precise therapeutic targeting. Presently a small number of defined probiotic bacteria have been shown to survive intestinal transit and seem effective adjuncts in the treatment of infectious or inflammatory diseases of the intestine. Bacteria based therapies must be validated by controlled clinical trials, which incorporate proof of probiotic activity and intestinal colonization.

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