Review

Biotherapy for and protection against gastrointestinal pathogenic infections via action of probiotic bacteria

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Abstract: The microbiota in the human intestine play an important function in human health and disease. Gastrointestinal infections by foodborne pathogens are a main cause of morbidity and mortality worldwide. Such infections can be caused by contaminated foods or other sources which come in contact with human intestinal epithelial cells. In recent years, probiotics have been recommended as alternative biotherapeutic agents against intestinal pathogenic infections. Two genera of probiotics, *Lactobacillus* and *Bifidobacterium*, are commercially valuable applications, several forms of which are available as capsules or in functional food products such as yogurt, fermented juices and sausages. Probiotics protect against gastrointestinal pathogenic infection via several mechanisms. These include production of antimicrobial substances, competition for nutrient substrates, competitive exclusion, enhancement of intestinal barrier function, and immunomodulation. Probiotic bacteria have been documented as being effective in biotherapeutic applications against gastrointestinal pathogens, e.g. *Helicobacter pylori*, *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, and rotaviruses. This alternative therapeutic application of probiotics to protect against gastrointestinal pathogenic infections may be of great importance for future medicinal use.

Keywords: biotherapy, foodborne pathogen, gastrointestinal infection, lactic acid bacteria, probiotic

INTRODUCTION

The human gastrointestinal tract harbours a complex and diverse ecosystem of microbiota or commensal microflora. It has been assumed that these microbiota range from 10^{12} to 10^{14} CFU/g of the luminal content [1]. There are in our body more than 2,000 different species, the majority of

which reside in the intestines [2]. Different communities of aerobic, facultative and anaerobic bacteria all constitute the gastrointestinal microbiota. The proportion of anaerobic bacteria gradually increases on going from proximal to distal areas; 99% of the inhabitants in the large intestine are anaerobes [3]. The diversity of microbiota species residing in the gastrointestinal tract is dependent upon the host's age, diet and health status [4]. Srikanth and McCormick [5] suggested that the intestinal mucosa may play a central role in host-microbiota-pathogen interactions. The human intestine is also an area which supports the energy metabolism and the immune function. Human microbiota may also play a critical role in disease and human health as suggested by Guarner and Malagelada [6] and Thirabunyanon et al [7]. Some cancers such as gastric cancer [8] and colon cancer [9] are also associated with the human microbiota and intestinal pathogenic infection. Probiotics have been promoted as new alternative biotherapeutic agents for human intestinal diseases. This report summarises the interactions between the host, microbiota and pathogens. It includes the use of probiotic bacteria as biotherapeutic agents in protection against, and treatment of, gastrointestinal infections.

FUNCTIONS OF MICROBIOTA IN THE GASTROINTESTINAL TRACT

The functions of microbiota in the human intestine consist of several main activities including metabolism, nutrition and disease protection (Table 1). Recent investigations using new techniques of molecular taxonomy have shed light on the composition, dynamics and ecology of the microbiota. Investigation of the diversity of human microbiota has revealed that this microbiota genome is at least 100 times larger than the human genome [10]. There are several types of microbial population in the human intestine such as *Lactobacillus* spp., *Bifidobacterium* spp., *Escherichia coli* and *Bacillus* spp. (Table 1). Three groups–aerobic, facultative and anaerobic bacteria–are indicated. However, the most abundant in the bacterial community are anaerobes, most of which (about 60-90%) are expressed in two divisions: the *Bacteroidetes* and the *Firmicutes* [11]. Eukaryotic fungi are also identified among the microorganisms inhabiting the intestinal tract [12].

The functions of intestinal microbiota may include diverse actions in the gastrointestinal tract including production of metabolites, nutritional fermentation and participation in the host's immune defense system. One role of human microbiota may involve maintaining nutritional homeostasis in the intestine. Nicholson and Wilson [13] suggested that several compounds produced from the microbiota co-metabolise nutrients with the host enzymes such as cytochrome P450 and conjugating enzymes in the liver. Ultimately these digested nutrients are absorbed by intestinal epithelial cells. The microbiota in the gastrointestinal tract may also produce or enrich metabolites such as glycans, amino acids, xenobiotics, vitamin K, folate and short-chain fatty acids (SCFA) [4, 10]. Starches are not easily digested by the human digestive system; however, the process is assisted by microbial fermentation. Turnbaugh et al. [14] indicated that the microbiota most able to produce SCFA are *Firmicutes* such as *Clostridium* spp. and *Bifidobacterium* spp. The primary metabolic end products of such fermentation are organic acids including SCFA such as butyrate, succinate and propionate [4, 15]. The functional roles of SCFA in colonic physiology may result in control of proliferation and differentiation of the intestinal epithelial cells [5, 16].

Table 1. Microbiota in the human gastrointestinal tract and their occurrence and/or possible functions

Microbiota	Occurrence and/or possible functions	Reference
Bacteroides spp. *Lactobacillus* spp. *Bifidobacterium* spp. *Streptococcus* spp. *Escherichia coli* *Clostridium* spp.	These bacteria originate from the birth canal and commence immediately after birth to colonise the gut; later they remain predominant in the gastrointestinal tract.	[3]
Enterococcus faecalis	Normally prevalent in healthy humans, but can cause infection under certain conditions.	[5]
Clostridium spp. *Bifidobacterium* spp.	High metabolic capacity, producing short-chain fatty acids (SCFA) within the lumen of the human gastrointestinal tract.	[14]
Pediococcus acidilactici MM33	The first human bacteriocin (pediocin)-producing strain which was found to be bactericidal against *Listeria monocytogenes*.	[34]
Lactobacillus johnsonii NCC 533	The original strain isolated from the human intestine that produces hydrogen peroxide and is effective in killing *Salmonella typhimurium*.	[36]
Lactobacillus rhamnosus IMC 501 *Lactobacillus paracasei* IMC 502	These antimicrobial strains could be used as health-promoting bacteria against harmful pathogens in humans.	[37]
Lactobacillus plantarum CS23	This strain induces potentially significant immunomodulatory activity in humans.	[38]

FUNCTION OF EPITHELIAL CELLS IN GASTROINTESTINAL TRACT

Protection of the host against intestinal pathogens is effected by the physical and chemical barriers of the gastrointestinal epithelium (Figure 1), which primarily consists of absorptive epithelial cells (enterocytes) [17]. Madara et al. [18] suggested that the human gut epithelium has a surface area of 300-400 m^2, comparable to the size of a tennis court. Epithelial cells lining the gastrointestinal tract constitute areas where contact is made between host and microbes. The structures of the apical surfaces of the epithelial cells are specialised and include microvilli, rigid intercellular junctions, and areas for ion secretion and mucus production [11]. Moreover, the microvillous tips of the epithelial cells have a surface coating of a mucous layer [17]. The intestinal epithelium also consists of several other cell types such as goblet cells, microfold (M) cells,

enteroendocrine cells, and Paneth cells. The intercellular junctional complexes are comprised of tight junctions, adherens junctions and desmosomes. The roles of these junctional complexes are to maintain the integrity of the epithelial barrier and to act as a physical barrier to prevent unwanted bacteria from entering the host [5].

The goblet cells secrete mucus in order to produce a mucous layer overlying the intestinal epithelium (Figure 1). This serves as a physical blockade protecting against harmful pathogens, which has been demonstrated with *Shigella flexneri* [19] and *Yersinia enterocolitica* [20]. The M cells differ from normal epithelial cells in that they lack microvilli on their apical surfaces. The primary roles of these M cells are in the transport of antigens, particles, macromolecules and microorganisms in the lumen through to the Peyer's patch and lymphoid tissue [21-22]. Enteroendocrine cells are hormone-secreting cells that sense the luminal environment and immediately react to secrete the correct peptide hormones such as cholecystokinin and secretin [5, 23]. The Paneth cells are another type of cells responsible for protection of the intestinal epithelium against pathogenic bacteria (Figure 1). They secrete certain antimicrobial peptides, e.g. alpha-defensins and cathelicidins. Paneth cells also produce several antimicrobial molecules including lysozyme, phospholipase A_2 and angiogenin-4 [23].

One important function of the intestinal epithelium is to create a surface where the host can sense the microbial microenvironment and generate protective responses against pathogens by producing an array of signalling molecules, e.g. chemokines and cytokines. These molecules stimulate the recruitment of leukocytes to initiate an early inflammatory response [5]. The host's immune response is expressed upon pathogenic infection; the specific recognition of molecular structures is determined by pathogen-associated molecular patterns. It has been proposed that the epithelial cells sense the microenvironment within the gut via pattern recognition receptors (PRR) including Toll-like receptors (TLRs) and nucleotide-binding oligimerisation domain (NOD) protein [24-25].

INFECTION PROCESSES OF GASTROINTESTINAL PATHOGENS

Enteric diseases are caused by several pathogens, notably *Salmonella* spp., *Escherichia coli*, *Shigella*, *Yersinia* and various other foodborne pathogenic strains such as *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Vibrio cholerae*. *Salmonella* is known to be implicated in human foodborne illnesses and often enters the food supply via contamination of food products such as poultry, pork, beef, dairy products and nuts, especially peanut and pistachio [26]. Other strains of foodborne pathogens also typically contaminate human foods.

There are two steps in gastrointestinal pathogenic infection. At the initial stage of the infection process, the pathogens attach themselves to the surfaces of intestinal epithelial cell structures consisting of glycoproteins and glycolipids, which serve as receptors for bacterial adhesion [27-28]. *Salmonella* spp. entering via the faecal/oral route can survive in and colonise the gastrointestinal tract. Adhesion to the epithelial cells is mediated by fimbriae or pili present on the bacterial cell surface [29]. During this entry step, bacterial pathogens can pass through the epithelial barrier, triggering a proinflammatory response [30]. During the second step of the infection process, direct cytotoxic injury, intracellular migration, and disruption of the epithelial tight junctions lead to mucosal infection and systemic spread of the disease [31-32].
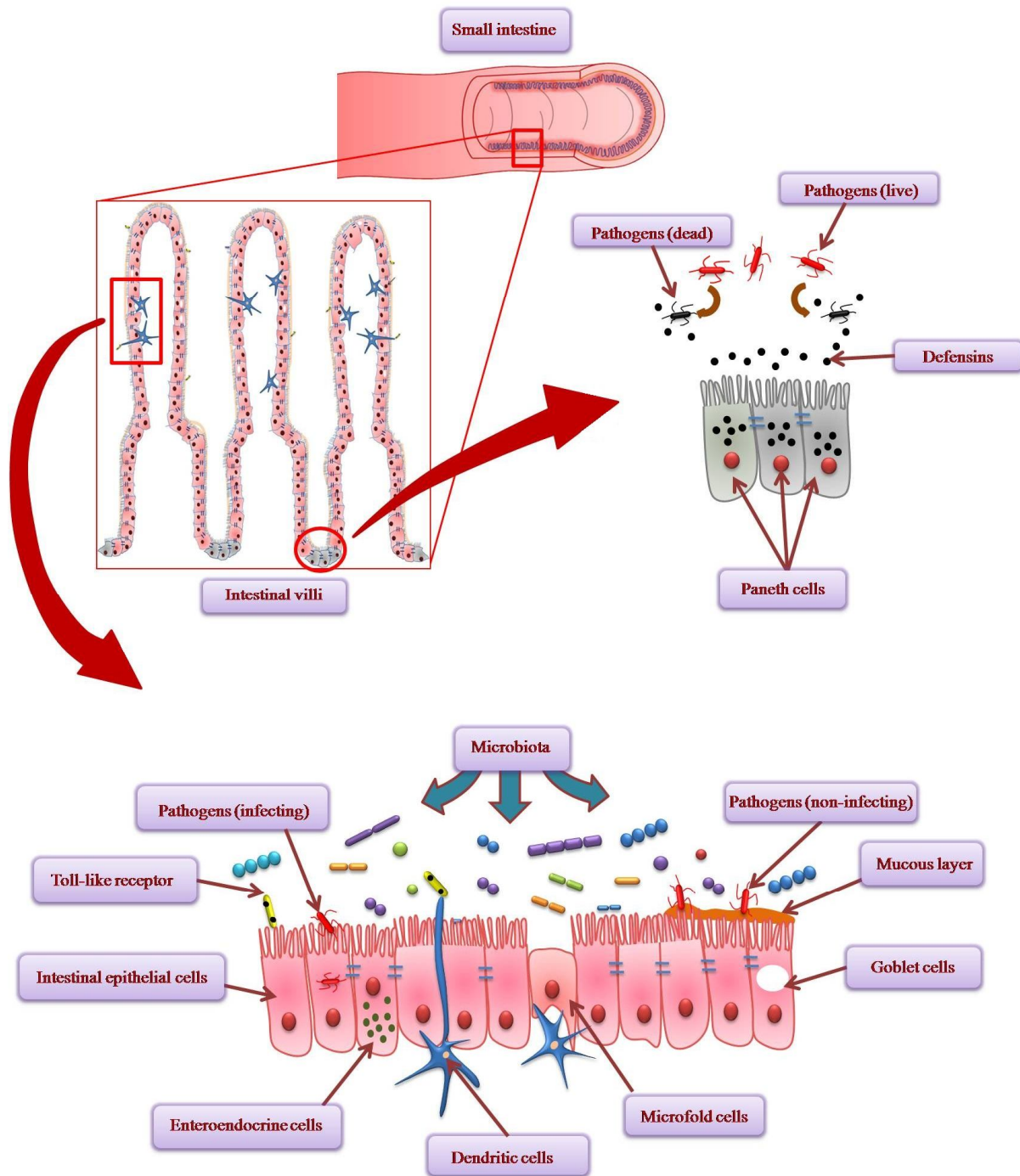
Figure 1. Functions of microbiota and epithelial cells in the lumen of human gastrointestinal tract. Intestinal microbiota are comprised of diverse groups (shown in different colours), i.e. aerobic, facultative and anaerobic bacteria, with different morphology such as rod and coccus. The intestinal epithelium consists of several cell types: intestinal epithelial, goblet, microfold, enteroendocrine, and Paneth cells. A mucous layer (brown) is a natural secretion produced by goblet cells and serves as a physical blockade protecting against pathogenic infection. Defensins (small black granules) are antimicrobial peptides secreted by Paneth cells against gastrointestinal pathogens.

THE CONCEPT OF PROBIOTICS

A probiotic is 'a live microbial food ingredient that is beneficial to health' [33]. Probiotics have recently received special attention on their application as an alternative approach to prevention of and therapy for several human gastrointestinal diseases [34-35]. Most of these potential probiotics are of human origin and are isolated from microbiota in the human gastrointestinal tract [34, 36-38]. Other sources are several human food products [39-41], which were also reported in our previous study of natural bacteria isolated from fermented milk products [7]. Recently, probiotic bacterial formulations have been developed for consumers in the forms of dietary supplements, yogurts, drinks and capsules. Two genera, *Lactobacillus* and *Bifidobacterium*, have been found to be excellent potential sources of bacterial probiotics. In addition, some species of *Enterococcus, Streptococcus* and *Bacillus* have also been suggested to have probiotic properties [7, 42-43].

Many criteria must be met to establish that a new bacteria strain is probiotic. These include non-pathogenicity, ability to inhibit the growth of pathogenic strains, tolerance for acid and bile salt conditions of the gastrointestinal tract, and ability to adhere to intestinal epithelial cells [7, 33, 44]. In vivo testing must be conducted in order to evaluate the probiotic activity in the body. If both in vitro and in vivo studies are successful, the probiotic bacteria can be used as a biotherapeutic agent in humans.

MECHANISMS OF PROBIOTIC ACTIONS AGAINST GASTROINTESTINAL PATHOGENIC INFECTION

Since the past decade probiotic biotherapeutic agents have increasingly been applied for prevention of and therapy for intestinal pathogenic infection. Consumption of probiotics may modulate the microbiota in the gastrointestinal tract and change their metabolic properties [45]. Many mechanisms have recently been postulated for these probiotic activities in the human gastrointestinal tract (Figure 2) [46-50].

Production of Antimicrobial Substances

One action of probiotics is that they can produce antimicrobial substances as direct antagonists against intestinal pathogens. Probiotics may exert their effective antagonistic activity alone or synergistically. Recent studies have indicated that the antagonistic activities against intestinal pathogens are produced by antimicrobial substances from several probiotic strains [7, 37-38]. These antimicrobial substances were found to range in size from small molecules to bioactive peptides. Bacteriocins are important ribosomally synthesised antimicrobial peptides which have been documented as possessing a good functional therapeutic activity against gastrointestinal pathogenic infection. These bacteriocins have been categorised into four classes: class-I bacteriocins are small peptides (which are also classified as lantibiotics) such as nisin; class-II bacteriocins are small, heat-stable peptides such as pediocin; class-III bacteriocins are large, heat-labile proteins such as helveticin J; and class-IV bacteriocins are complex bacteriocins [3, 51-52]. Millette et al. [34] indicated that pediocin, the bacteriocin secreted by *Pediococcus acidilactici* MM33 isolated from the human gut, was bactericidal against *Listeria monocytogenes*. Reuterin, an antimicrobial compound produced by some strains of *Lactobacillus reuteri*, may act as an antagonist against enteric pathogens [35, 53]. A study by Pridmore et al. [36] showed that the human intestinal
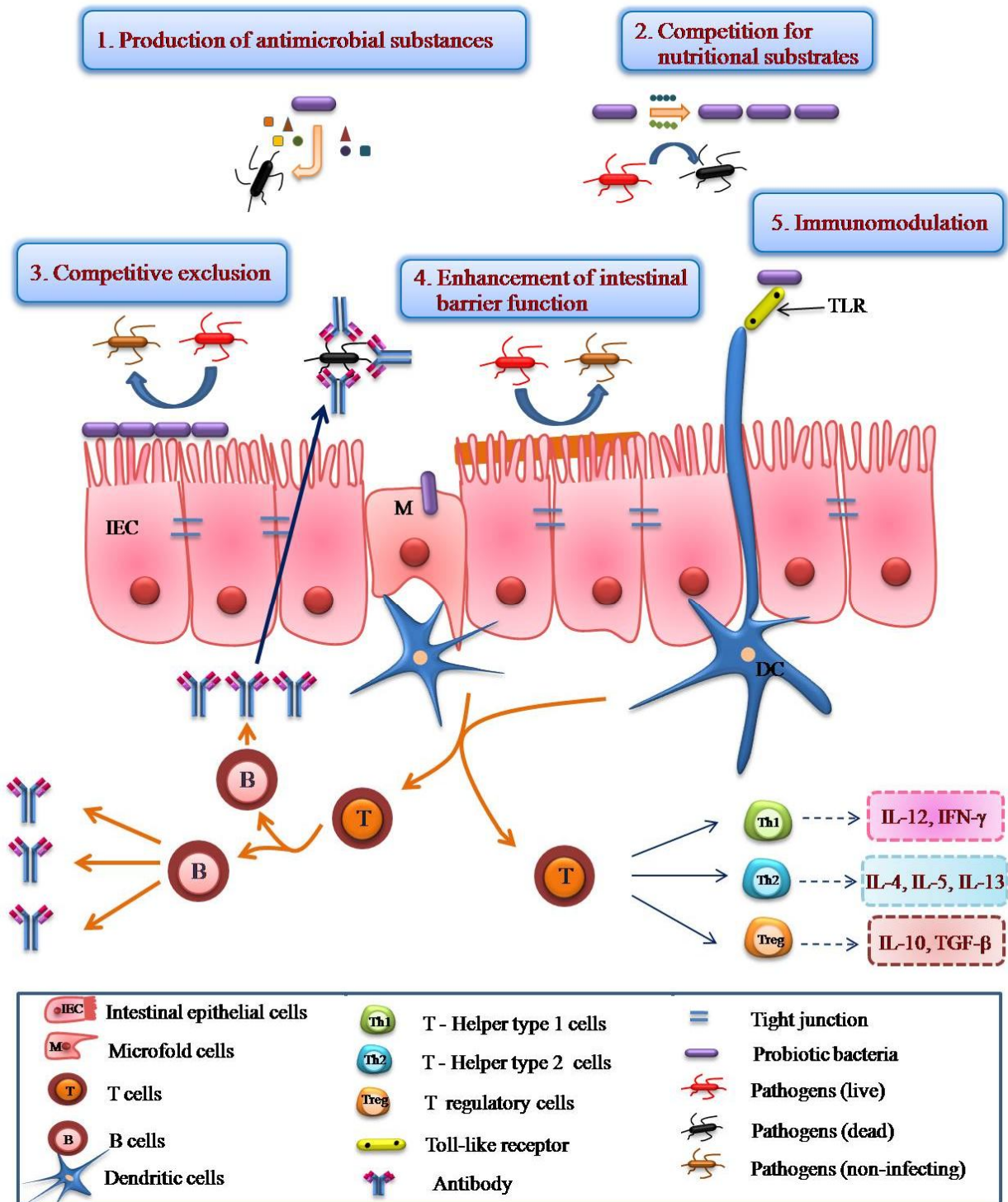
Figure 2. Schematic illustration of postulated mechanisms of probiotic bacterial actions against gastrointestinal pathogenic infection: (1) production of antimicrobial substances; (2) competition for nutritional substrates; (3) competitive exclusion; (4) enhancement of intestinal barrier function; and (5) immunomodulation.

probiotic strain of *L. johnsonii* NCC533 (La1) can produce hydrogen peroxide that is effective in killing *Salmonella typhimurium*.

Other metabolites from probiotics are potential antimicrobial substances that can protect against intestinal pathogenic infection. It has been found that five strains of *Pediococcus* spp. produce several factors that inhibit the growth of *Listeria monocytogenes*, notably hydrogen peroxide, lactic acid, exopolysaccharides, and proteolytic activity [39]. Probiotics which can produce metabolites such as acetic and lactic acids may lower the pH in the intestine. This lowering of pH results in inappropriate environmental conditions for pathogenic growth. An in vitro study by Ridwan et al. [54] showed that the antimicrobial activity of a multi-species probiotic product (Ecologic 641) may be exerted by the production of organic acids. Likewise, a biosurfactant produced from *Lactobacillus paracasei* was shown to have bactericidal activity that inhibited the growth of several pathogens [55].

Competition for Nutritional Substrates

The enteric probiotic population in the gastrointestinal tract may increase after consuming nutrients. Thus, competition for nutritional substrates amongst probiotics, intestinal pathogens and microbiota may occur. Hojo et al. [56] suggested that *Bifidobacterium adolescentis* S2-1 can better utilise vitamin K and inhibit the growth of *Porphyromonas gingivalis* by competing for the growth factor. In an animal model of germ-free mice colonised with human baby microbiota, the diverse metabolic profiles have been investigated after exposure to a probiotic strain of either *Lactobacillus paracasei* or *Lactobacillus rhamnosus*. These probiotic treatments may alter a diverse range of pathways which include the metabolism of amino acid, methylamines and SCFA [57]. Similarly, Stanton et al. [58] produced biogenic metabolites such as vitamins, fatty acids and bioactive peptides which were marked through applying probiotics in fermented functional foods. The biogenic metabolites may act as a growth substrate for selected compounds with different probiotics, intestinal pathogens or microbiota.

Competitive Exclusion

Probiotics can eliminate pathogens at the adhesion and infection site of epithelial cells in the human intestine by competitive exclusion. Infection begins with the binding of the pathogen to intestinal epithelial cells through the interaction between bacterial lectins and carbohydrate moieties of glycoconjugate receptor molecules on the intestinal epithelial cell surface [47]. Mukai et al. [59] suggested that the binding ability of *Bifidobacterium bifidum* and *Lactobacillus reuteri* to intestinal glycolipids may play an important role in their ability to adhere to the epithelial surface of the intestine. Competition study by Ramiah et al. [60] indicated that *Lactobacillus plantarum* 423 is able to colonise intestinal epithelial cells, thus preventing the adhesion of pathogenic *Clostridium sporogenes* and *Enterococcus faecalis*. These findings were similar to the author's unpublished data which indicated that a novel probiotic strain of *Bacillus subtilis* NC11 has a protective activity against *Salmonella enteritidis* infection of intestinal epithelial cells. Thus, probiotic actions against pathogenic infection can be through competitive adhesion and/or blocking of the penetration of pathogens at the infection site of intestinal epithelium cells by competing for the glycoconjugate receptors.

Enhancement of Intestinal Barrier Function

The pathophysiology of intestinal pathogenic infection displays a disruption of epithelial barrier function and a loss of tight junction formation in the intestinal epithelium cells [61]. These phenomena can increase the pathogenic or enterotoxic permeability of the mucosa wall. Probiotics have been promoted for their enhancement of intestinal barrier function by impeding the translocation and attachment of pathogenic bacteria to the intestinal epithelium [62]. Khailova et al. [63] showed in a rat model that administration of *Bifidobacterium bifidum* may have a protective effect through regulation of the main components of the mucous layer and improvement of intestinal integrity. Similarly, Mennigen et al.[64] suggested that the probiotic mixture VSL#3 can protect the epithelial barrier in a mouse model of acute colitis by maintaining tight junction protein expression and preventing the increase of apoptotic ratio.

Immunomodulation

The role of intestinal epithelial cells is associated with immunomodulation through complex interactions between immune cells and probiotics, triggering a cascade of appropriate innate or adaptive immune defense responses [47, 65]. The production of pro-inflammatory or anti-inflammatory cytokines by human peripheral blood mononuclear cells is challenged with *Lactobacillus plantarum* L2. It was found that this bacterium can induce interleukin (IL)-10 but only low levels of the pro-inflammatory cytokines TNF-alpha, IFN-gamma and IL-12. During an in vivo study, a significant increase in CD19-positive cells in the ileum was found after a daily feeding of *L. plantarum* L2 in rats [66]. Amit-Romach et al. [67] indicated that administration of the probiotic strain *Lactobacillus* GG and a mixture of *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium lactis* in rats may reduce the expression of pro-inflammatory cytokines TNF-alpha and IL-6. Martínez-Cañavate et al. [68] suggested that consumption of probiotic products by children may result in enhanced innate immunity through a significant increase in natural killer cells and other specific immune factors that may improve their health status.

PROBIOTICS AS BIOTHERAPEUTIC AGENTS IN GASTROINTESTINAL PATHOGENIC INFECTIONS

Enteric pathogenic infections are a main cause of morbidity and mortality worldwide. It has been recorded that severe diarrhea and dehydration caused the deaths of 1,575,000 children under the age of five in 2006−15% of the 10.5 million deaths per year of children in this age group [69]. The enteric pathogens, notably *Helicobacter pylori*, *Salmonella enteritidis*, *S. typhimurium*, *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes*, *Clostridium difficile*, *Campylobacter jejuni* and *Vibrio cholerae*, cause a variety of human diseases including gastroenteritis, peptic ulcer and diarrhea. These pathogens are also associated with gastric [8] and colon cancers [9]. Probiotics have been applied as alternative and biotherapeutic agents for prevention of and therapy for gastrointestinal pathogenic infections as described below.

Helicobacter pylori

Pathogenic infection by *H. pylori* can lead to chronic gastritis and peptic ulcer and increase the risk of gastric cancer [70]. *H. pylori* infection is currently treated with a proton pump inhibitor

combined with clarithromycin and amoxicillin or metronidazole [71]. Although the use of antibiotics for treatment is efficient, it is expensive and has many side effects including stimulation of antibiotic resistance in intestinal pathogens [72]. As a result, alternative application of probiotics for prevention of and therapy for *H. pylori* has been investigated. Pathogenic *H. pylori* are known to produce urease, which can hydrolyse urea to ammonium species, resulting in elevated pH in the stomach and promoting adhesion of microorganisms [73]. Thirabunyanon et al. [7] found that the potential probiotics, *Enterococcus faecium* RM11 and *Lactobacillus fermentum* RM28, isolated from fermented dairy products could inhibit the growth of pathogenic *H. pylori*. In an investigation, 14 patients infected with *H. pylori* received milk containing the probiotic *Lactobacillus casei* Shirota strain continually for 6 weeks. The results showed that urease activity declined in 64% of the patients who consumed the fermented milk, as compared with 33% for the control group [74]. Similar results were obtained by Myllyluoma et al. [75], who concluded that decreasing urease and gastrin-17 activities were found in *H. pylori*-infected patients who consumed a probiotic combination of *Lactobacillus rhamnosus* GG, *L. rhamnosus* LC705, *Propionibacterium freudenreichii* JS and *Bifidobacterium lactis* Bb12 for 8 weeks.

The suppression of *H. pylori* binding to the glycolipid receptors by the probiotic *Lactobacillus reuteri* has been reported [76]. Lin et al. [77] proposed that lactic acid bacteria isolated from commercial food products can inhibit *H. pylori* infection at the adhesion sites of human gastric epithelial AGS cells. Sgouras et al. found that *Lactobacillus casei* Shirota was highly effective in reducing *H. pylori* colonisation in the antrum and body mucosa in a mouse model [78] while *Lactobacillus gasseri* OLL2716 was shown to be effective against *H. pylori* infection in children [79]. Similarly, Wang et al. [80] indicated that regular consumption of yogurt containing *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12 may be effective in inhibiting *H. pylori* infection in humans. The outcome of using two combined probiotic strains of *Bacillus subtilis* and *Streptococcus faecium* for *H. pylori* eradication in patients were observed. These actions of the probiotic group were found to have a higher eradication rate (83.5%) than that of the control group (73.3%) [81].

Salmonella spp.

Salmonella is a major foodborne pathogen normally found in many food products. It causes many human diseases such as gastroenteritis, enteric fever, bacteremia, focal infections and enterocolitis. Human salmonellosis has become an important international public health and economic issue [82-84]. Continual use of antimicrobial agents for treatment of salmonellosis may result in the emergence of antibiotic-resistant strains of *Salmonella*. This multi-drug resistance has caused great public health concern [85-86].

The study of Thirabunyanon et al. [7] showed that lactic acid bacteria isolated from dairy products suppress the growth of *Salmonella typhimurium* and *S. enteritidis* [7]. Maragkoudakis et al. [87] observed that two food-derived probiotics, *Enterococcus faecium* PCD71 and *Lactobacillus fermentum* ACA-DC179, when co-cultured in raw chicken meat, could protect it against *Salmonella enteritidis* contamination by inhibiting its growth. A protective role of *Lactobacillus acidophilus* Bar13, *L. plantarum* Bar10, *Bifidobacterium longum* Bar33 and *B. lactis* Bar30 strains against *Salmonella typhimurium* infection of intestinal epithelial cells has been proposed [88]. Similarly, Thirabunyanon et al. found that a novel probiotic *Bacillus subtilis* NC11 strain has a protective

activity against *Salmonella enteritidis* infection of intestinal epithelial cells (unpublished data). Fayol-Messaoudi et al. [40] showed that the probiotic *Lactobacillus plantarum* ACA-DC287 strain isolated from Greek cheese can inhibit the adhesion of *Salmonella typhimurium* to intestinal epithelium cells. When mice infected with *S. typhimurium* took this probiotic, it resulted in a decrease in the levels of *Salmonella* in the intestinal tissues and contents. *Lactobacillus fermentum* ACA-DC179 was found to exert a protective effect against *S. typhimurium* infection in mice [89] while two *Lactobacillus* strains, LAP5 and LF33, showed significant antagonistic effects against *S. typhimurium* invasion of internal organs such as liver and spleen in mice that were fed the lactic acid bacteria daily for 7 consecutive days [90]. Similarly, Chiu et al. [41] found that *Pediococcus pentosaceus* MP12 and *Lactobacillus plantarum* LAP6 are able to inhibit *Salmonella* invasion in mouse liver and spleen. In another study, mice pre-fed for 7 days with milk containing *Lactobacillus casei* (probiotic dahi) prior to challenging with *Salmonella enteritidis* showed increasing production of IL-2, IL-6 and IFN-gamma, whereas IL-4 decreased in splenic lymphocytes, indicating protection against *S. enteritidis* infection by enhancement of innate and adaptive immunity [91].

Escherichia coli

Diarrheagenic *E. coli* is known to be the cause of various forms of diarrhoea and is classified into six categories, namely enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAggEC) and diffusely adherent *E. coli* (DAEC) [92-93]. Certain strains of EHEC are highly infectious pathogens that produce one or more Shiga toxins which induce gastrointestinal diseases such as diarrhoea, hemorrhagic colitis and life-threatening hemolytic uremic syndrome (HUS) in humans [94-95]. It is known that outbreaks of EHEC with serotype O157:H7 continually occur worldwide and pose a serious global health threat [96]. EHEC likely evolved from an EPEC strain; this enables EHEC to produce lesions on host intestinal epithelial cells, thus reducing intestinal epithelial barrier function [97]. EAggEC infection is associated with childhood [98] and adult diarrhoea [99] such as travellers' diarrhoea, pediatric diarrhoea and persistent diarrhoea [100]. These EAggEC strains have been observed in weaning foods, infant feeding bottles, milk and water [101]. Limited use of antibiotics for treating *E. coli* infection and alternative therapies such as application of probiotics are recommended.

Probiotic *Lactobacillus acidophilus* RY2 strain isolated from faeces of healthy infants can inhibit EAggEC adhesion to intestinal epithelial cells, thus preventing pathogenic colonisation and infection [102]. Similarly, Ostad et al. [103] concluded that the probiotic *L. acidophilus* in both live and heat-inactivated forms isolated from neonatal faeces decreases the adhesion of *E. coli* to intestinal epithelial cells. Miyazaki et al. [93] demonstrated that a probiotic strain of *Enterococcus faecium* has bactericidal effects on EAggEC by inducing membrane damage and cell lysis. Protection of the tight junction of intestinal epithelial cells against EHEC-induced damage has been found via the activity of probiotic *Bifidobacterium lactis* 420 strain [104]. Mangell et al. [105] pre-fed rats with probiotic *Lactobacillus plantarum* 299v strain in drinking water and then challenged them with an *E. coli*-induced increase in intestinal permeability. The results showed that this probiotic strain can exert a protective effect. A comparison of probiotic feeding with *Bifidobacterium thermacidophilum* RBL71 for 7 days before and after infection with EHEC *E. coli* O157:H7 in mice was investigated.

The effects were greater in the pre-challenged group compared to the after-treatment group, resulting in increased feed intake and weight gain and lower faecal levels of *E. coli* O157:H7 [106].

Listeria monocytogenes

L. monocytogenes has been found to be a contaminant in various raw and processed foods such as beef, pork, sausages, milk, dairy products, vegetables and seafood products [107-108]. It causes listeriosis, a foodborne pathogenic illness that primarily infects pregnant women, newborns and elderly or weakened individuals [109]. *Listeria* has also been implicated as the cause of septicemia, spontaneous abortion and even death of infected individuals [110]. The mortality rate of this illness may reach 20-30%, making it a serious public health menace [107]. *L. monocytogenes* is known to tolerate environmental stresses including variations in pH, temperature and osmolarity [111]. Because it can survive in foods for long periods of time, it has been implicated in outbreaks in meat and dairy products [112-113].

Infection by *L. monocytogenes* may translocate from the gastrointestinal tract to other organs such as liver, spleen, central nervous system and placenta [114]. Several biotherapeutic agents for *L. monocytogenes* infection have been investigated. De Waard et al. [115] demonstrated that rats fed *Lactobacillus casei* Shirota YIT9029 strain continuously for 3 days before being infected with *L. monocytogenes* show reduced levels of the pathogen in the faeces and several organs, i.e. stomach, caecum, spleen and liver. Corr et al. [116] observed the anti-infective activity of *Lactobacillus salivarius* UCC118, a strain of human origin that produces Abp118 bacteriocin which can protect against *L. monocytogenes* infection in mice. In another study, after orally feeding *Lactobacillus plantarum* to mice continuously for 30 days and then challenging by intravenous infection with a clinical strain of *L. monocytogenes*, it was found that the administration of *L. plantarum* reduces pro-inflammatory interleukin (IL-1 beta and IL-6) production, implicating the host protection against *L. monocytogenes* [117]. Similar results were found in mice treated with *Lactobacillus delbrueckii* var. *bulgaricus* UFV-H2b20 and challenged with *L. monocytogenes*. The mice were more resistant to this pathogenic infection, as registered by mortality rates and number of bacteria in spleen and liver. They also showed increasing production of inflammatory cytokines (interferon-gamma and tumor necrosis factor-alpha) and nitric oxide [118].

Clostridium difficile and rotavirus

Evaluation of potential probiotics for their ability to protect against infection by other intestinal pathogens has also been undertaken. Effective probiotic treatments of *C. difficile* infection which causes gastrointestinal illness have been proposed [119-122]. Protection against rotavirus infection which is a leading cause of gastroenteritis, especially in young children, has also been investigated [123-124].

CONCLUSIONS

Recently, human diseases and probiotic bacteria have become interrelated fields of investigation through the association with gastrointestinal infections from foodborne pathogens that are known to be a main cause of morbidity and mortality worldwide. Hence, many studies are now in progress on the applicability of probiotic bacteria as an alternative biotherapeutic treatment for, and

protection against, gastrointestinal pathogenic infections. Probiotic bacteria are derived from human microbiota; since they are of human origin, they may have key features as primary sources for human disease therapies. New sources which originate from fermented foods are also significant for both functional food development and alternative biotherapies. One important limitation is that only one kind of probiotic bacteria may not exert protection against all harmful strains that cause gastrointestinal pathogenic infections. Therefore, effective investigations of individual strains of probiotic bacteria and also of new formulations that combine several probiotic activities in challenging certain gastrointestinal pathogens—in vitro, by cell culture, and in animal models as well as in humans as a final evaluation—are necessary before a biotherapeutic application. Biotherapy with probiotic bacteria for gastrointestinal pathogenic infections may modulate functions of the microbiota and intestinal epithelium in the gastrointestinal tract, resulting in many documented action modes such as antimicrobial production, nutritional substrate competition, competitive exclusion, intestinal epithelial function, and immunomodulation. The present investigations of this alternative biotherapeutic application of probiotics to protection against gastrointestinal pathogenic infections may be of great importance for both present and future medicinal use.

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